MONOGRAPHS ON BIOCHEMISTRY

EDITED BY

R. H. A. PLIMMER, D.Sc.

AND

F. G. HOPKINS, M.A., M.B., D.Sc., F.R.S.

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THE FATS

BY

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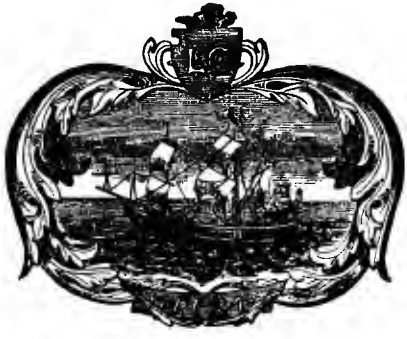


NEW IMPRESSION

LONGMANS, GREEN, AND CO.

39 PATERNOSTER ROW, LONDON

NEW YORK, BOMBAY, AND CALCUTTA

1913

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE.

THERE are many who study Physiology without appreciating how far the chemistry of the fats has been advanced by technical and scientific chemists. At the same time, there may be chemists, versed in the chemistry of fats, who do not appreciate all that this subject may mean to the biologist. This small book, which is aimed to reach both these classes of readers, and so runs the risk of missing both, must be considered to have attained its object if it proves useful to either. For the field of biochemical work to which it refers needs workers, who may be either physiologists who have trained themselves chemically, or chemists who are alive to the legitimate aspirations of biology.

J. B. L.

CONTENTS.

INTRODUCTION.

THERE is a large group of substances widely distributed through the animal and vegetable kingdoms which have one property in common, that they contain in their chemical constitution and are mainly composed of the radicals of fatty acids, principally and generally only the higher ones. These compounds with but few well-attested exceptions form a group by themselves that does not overlap the borders of the other groups of substances, the proteins and carbohydrates, which together with them constitute the material in which the phenomena of life are manifested. They are at least as sharply defined a group as either of these, and they hardly can be said to have less physiological significance. And yet there is no one comprehensive name to include them all. The term fat has a precisely defined chemical meaning which restricts its application to a certain number only of the substances of this group, and yet many of these substances that are in the strict chemical sense fats have on account of their physical properties to be spoken of as oils, while some are generally known as waxes: of the compounds, on the other hand, that are not in the strict sense fats, some have had assigned to them the term "wax," a term for which also a chemical definition has been devised that does not correspond to the ordinary meaning of the word, while for a large class, the physiological importance of which is daily coming more into evidence, no better general name has been proposed than "lipoids," which is at once a cloak for ignorance and an indefinable limbo into which any one can thrust anything of which he knows little or nothing, including often what is not a compound of any fatty acid at all.

Of these compounds of fatty acids occurring in nature the commonest are esters of the triatomic alcohol glycerol. These are fats when they are solid at the ordinary temperature and oils when they are liquid. Such esters may contain one, two or three fatty acid radicals, the mono-, di- or triglycerides as they are respectively called; for instance the esters formed by butyric acid with glycerol are:—

1. the α and β monobutyrins—

$$\alpha\begin{cases}CH_2 . OOC . C_3H_7\\ |\\ CH . OH\\ |\\ CH_2 . OH\end{cases} \qquad \beta\begin{cases}CH_2 . OH\\ |\\ CH . OOC . C_3H_7\\ |\\ CH_2 . OH\end{cases}$$

Of these the α form must clearly be capable of occurring in two stereoisomeric optically active modifications:—

2. the $a\beta$ and the aa dibutyrin—

$$a\beta\begin{cases}CH_2 . OOC . C_3H_7\\ |\\ CH . OOC . C_3H_7\\ |\\ CH_2 . OH\end{cases} \qquad \text{and } aa\begin{cases}CH_2 . OOC . C_3H_7\\ |\\ CH . OH\\ |\\ CH_2 . OOC . C_3H_7\end{cases}$$

the $a\beta$ form being also capable of stereoisomerism :—

3. tributyrin—

$$\begin{array}{l}CH_2 . OOC . C_3H_7\\ CH . OOC . C_3H_7\\ CH_2 . OOC . C_3H_7\end{array}$$

But in one and the same diglyceride or triglyceride molecule the fatty acid radicals may be not all the same as in the above instances; there may be two different radicals in a diglyceride and two or even three different ones in a triglyceride; and then other stereoisomeric modifications become possible in the case of the *aa* diglycerides and also of the triglycerides.

When it is remembered that glycerides of a very large number of fatty acids occur in nature, it is clear that the number of arrangements of these in glycerol esters must be large indeed.

Again, when a glyceride contains less than three fatty acid radicals the place of the third or possibly of the second and third may be taken by an acid of a different kind. In lecithine, for instance, there are two fatty acid radicals, and the third hydroxyl group of the glycerol is coupled by an ester union with phosphoric acid. And there are many other compounds in which there is reason for supposing that phosphoric acid and fatty acids are coupled with the same glycerol molecule. Since phosphoric acid is a tribasic acid, the acid hydroxyl groups which are not engaged by the glycerol in such a compound are free to enter into combination with other groups, alcohols or bases; as, for instance, in lecithine, with the alcoholic base choline.

Then, besides glycerol, there are several other alcohols that are found associated with fatty acids, and some of these are very widely distributed, if only in relatively small amounts.

Lastly, it must be remembered that fats and oils as they occur in nature are never single chemical substances, seldom indeed entirely composed of glycerides. In any fat or oil there is a mixture of several glycerides with varying quantities of cholesterol or substances related to it and often free fatty acids or soaps as well.

These considerations show that the number of compounds of fatty acids which have to be dealt with in biological chemistry, and for which we have no single comprehensive name, is very large; and also that the precise constitution of any one such compound may, even when it is not complicated by the presence of phosphoric acid, be difficult to determine, whereas, where this complication does exist, the difficulty is bound to be very greatly increased. In fact at the present time these more complicated compounds of the fatty acids are among the most obscure of those with which animal or vegetable chemistry has to deal, more obscure and indefinite even than many of the proteins.

The plan adopted in the following biochemical study of the compounds of fatty acids is first to review the properties and characteristics of the various fatty acids that are found in animals or plants, then those of the alcohols and their fatty acid esters. In the second section the methods in use in the laboratory for obtaining and determining the amount of compounds of fatty acids present in any given material will be dealt with, principally from the point of view of the student of animal physiology. In the third section the methods that have served hitherto for the purpose of examining and determining the character of the compounds obtained will be described, and finally, in the fourth section some points will be discussed in the physiology of the compounds dealt with in the previous sections.

NOTE ON TERMINOLOGY.

The following terms will be used in these pages :—

Phospholipines, to denote compounds of fatty acids that contain phosphorus and nitrogen.

Galactolipines, to denote compounds of fatty acids that contain nitrogen and galactose.

Lipines, to denote compounds of fatty acids containing nitrogen but no phosphorus or carbohydrate group.

The phospholipines correspond to the phosphatides of Thudichum, and include lecithine, kephaline, cuorine and sphingomyeline, and other still less completely defined substances.

The galactolipines correspond to the cerebrins, cerebrosides or cerebrogalactosides of various authors.

The lipines correspond to the amido lipotides and cerebrin acids of Thudichum, and include certain other substances described by others.

The practice of dividing up the "phosphatides" into mono- and diamino mono- or di-phosphatides has less to be said for it than at first sight appears. The resulting names convey no more than is conveyed when the ratio N : P is given ; they cannot be remembered unless this ratio is remembered. Objections to the use of the term phosphatides for any of these substances are given in a later section.

CHAPTER I.

THE SUBSTANCES THAT ENTER INTO THE COMPOSITION OF FATS.

A. THE FATTY ACIDS.
B. GLYCEROL AND THE GLYCERIDES.
C. OTHER ALCOHOLS AND THEIR FATTY ACID ESTERS.
D. PHOSPHOLIPINES, GALACTOLIPINES AND LIPINES.

A. THE FATTY ACIDS.

THE acids with which we are concerned belong to more than one series; members of the following series are known to occur in fats and waxes :—

I. The saturated fatty acids with the general formula $C_nH_{2n}O_2$, the series taking its name from the acid in which $n = 2$, acetic acid.

II. The unsaturated fatty acids among which members of the following series are known to occur :—

(*a*) Acids with the general formula $C_nH_{2n-2}O_2$, the series taking its name either from acrylic acid ($n = 3$) or oleic acid ($n = 18$).

(*b*) Acids with the general formula $C_nH_{2n-4}O_2$, the series taking its name from linoleic acid ($n = 18$).

(*c*) Acids with the general formula $C_nH_{2n-6}O_2$, the series taking its name from linolenic acid ($n = 18$).

(*d*) Acids with the general formula $C_nH_{2n-8}O_2$.

III. Saturated oxy-acids $C_nH_{2n}O_3$ rarely occur.

IV. Unsaturated oxy-acids: the only acids known have the formula $C_nH_{2n-2}O_3$ ($n = 18$), and are named after the one occurring in castor oil, ricinoleic acid, from which the others are artificially prepared.

V. Saturated dioxy-acids: the only acid known to occur in fats, has the formula $C_nH_{2n}O_4$ ($n = 18$); it is found as a glyceride forming 1 per cent. of castor oil.

VI. Saturated dibasic acids: the only acid known to occur in fats, has the formula $C_nH_{2n-2}O_4$ ($n = 22$), and occurs as a glyceride in the vegetable product known as Japan wax.

VII. Certain cyclic acids are described as occurring in the vegetable chaulmoogra oil.

I. The Saturated Fatty Acids: $C_nH_2O_2$.

The acids of this series, up to and including the acid containing ten carbon atoms, capric acid, are fluids at the ordinary temperature; those members of the series that contain more than ten carbon atoms are solids, the melting point of which rises with the molecular weight.

The lowest members containing four or less than four atoms of carbon are miscible with water in all proportions; as the molecular weight increases beyond this, the solubility of the acids rapidly diminishes to zero. Caproic acid is slightly soluble in cold water, caprylic acid requires 400 parts of boiling water to dissolve it, whereas capric and lauric acids dissolve only in traces even in boiling water, and the acids higher in the series still are practically insoluble in water.

The physical property, however, which is most useful in the grouping of these acids is one common to all the lower members of the series, from capric acid down, that of being easily distilled with steam; these acids are the volatile fatty acids. The acids next above capric acid in the series, myristic and lauric, pass over in a current of steam merely in traces, and the acids higher still barely even in traces. By reference therefore to this property the acids are fairly sharply divided into two groups, the volatile and the non-volatile acids.

With increasing molecular weight the boiling point of the acids also rises, at atmospheric pressure by increments of about 20° C. with each additional carbon atom, from formic acid (B.P. 100·8) to capric acid (B.P. 268). Beyond this temperature the acids that have a higher boiling point are decomposed, and their boiling points therefore can be determined only at lower pressures. In an absolute vacuum lauric acid boils at 102°, stearic acid at 155°; at 15 mm. pressure stearic acid boils at 232°.

The volatile acids up to and including butyric acid smell pungently acid; caproic, caprylic and capric acids have, as the names imply, offensive goatlike odours.

The solidifying point of melted acids is lower than the melting point, and generally can be more sharply determined.

The melting point of mixtures of the acids is lower than would be calculated from the melting points of the pure acids composing the mixture. In this respect the fatty acids behave similarly to the metals in alloys. They form also eutectic mixtures, tending to crystallise in certain fixed proportions with constant melting and solidifying point. Such a mixture of stearic and palmitic acids in molecular proportions has frequently been described as a chemical entity, margaric acid, which is now believed not to occur in nature.

It is a remarkable fact, which has frequently been commented on, that the fatty acids that occur in nature, excluding one or two of the lowest members of this series, are almost without a well-certified exception acids that contain an even number of carbon atoms; and also with very few and rare exceptions they are the normal acids, with chains of carbon atoms that do not branch.

Many of the most important properties of the acids are contained in the table of physical constants of the acids appended, and in the brief review of the several individual acids that here follows, note will be made only of a few additional points that may be most frequently of value in the study of fats:—

Formic acid occurs in sweat, urine and meat juice, and in the bodies of ants, especially the red ant.

When heated with strong sulphuric acid it breaks up into carbon monoxide and water. It is readily oxidised by metallic oxides, *e.g.*, HgO, which is reduced to Hg_2O and the metal; and by contact with rhodium it is converted into carbon dioxide and water.

All its salts are soluble in water with the exception of the mercurous salt, which spontaneously decomposes by reduction of the mercurous oxide to the metal and formation of carbon dioxide.

It is recognised by the fact that neutral solutions of its salts reduce silver nitrate, and when treated with neutral ferric chloride give a red colour and, on warming, a yellow precipitate of basic salt.

Acetic acid occurs in sweat, muscles, the liver, fæces, urine and sometimes in the stomach.

The silver salt may be precipitated on adding silver nitrate to a fairly concentrated solution of an acetate, and can be crystallised in fine needles from hot water; the crystals contain 64·65 per cent. silver.

It is recognised by the smell of the ethyl ester formed when ethyl alcohol is added to a solution of the acid or its salts, and the mixture treated with strong sulphuric acid.

Propionic acid occurs in sweat.

It can be salted out of its aqueous solutions by means of calcium chloride like butyric acid.

All its salts are soluble, but the silver salt the least soluble.

Its basic lead salt is easily soluble in cold but not in hot water, in which respect it differs from the salts of formic, acetic and butyric acids.

Normal butyric acid occurs in sweat, fæces and urine, and in the form of glycerides, to the extent of about 6 per cent., in butter.

Freshly distilled it smells like acetic acid, diluted with water it has a rancid smell. Miscible with water in all proportions, it can be salted out like propionic acid by means of calcium or sodium chloride.

Its salts are easily soluble in water, with the exception of its silver salt, of which 1 part dissolves in about 200 of water at 14 ; its mercurous and lead salts are also comparatively insoluble.

Calcium butyrate, crystallising with 1 molecule of water, is soluble in alcohol, and is more soluble in cold than in hot water; at 0° C. 100 parts of water dissolve 19·4 parts of this salt, of which 23 per cent. separates out on heating to 70° C., the temperature at which it is least soluble. The barium salt crystallises with 4 molecules of water. The smell of the ethyl ester, resembling that of pine-apples, is characteristic.

Isobutyric acid occurs in fæces and among the products of bacterial action upon proteins.

It is less soluble in water (1 in 5) than the normal acid.

Its calcium salt crystallises with 5 molecules of water, and unlike the normal butyrate, is more soluble in hot than in cold water.

Valerianic acid is said to be formed by certain bacteria from lactic acid; its calcium salt resembles that of butyric acid in its solubility.

Isovalerianic acid (isopropylacetic acid) was stated by Chevreul (1817) to occur as a glyceride in the blubber of porpoises. It occurs among the products of protein decomposition.

It dissolves in 23·6 parts of water at 20° C., and is thrown out of solution on adding calcium chloride.

Its salts when thrown upon water show a rotary movement; those of the alkaline earths and of the alkalis are soluble.

Normal caproic acid, $C_6H_{12}O_2$, occurs in fæces and in the butyric fermentation of sugar, and in the form of glycerides, to the extent of about 1·2 per cent., in butter, and also in cocoa-nut and palm-nut oils.

It is slightly soluble in water.

Its calcium salt crystallises with 1 molecule of water, 4·4 parts of the anhydrous salt dissolve in 100 parts of water at 21° to 22° C.

The barium salt crystallises with 2 molecules of water, and is rather more soluble (11·1 parts in 100 of water at 10·5° C.).

Crystals of the zinc salt are precipitated when caproic acid is poured on to a solution of zinc acetate; by this it may be distinguished from butyric and valerianic acids.

Caprylic acid, $C_8H_{16}O_2$, occurs in sweat and in the form of glycerides in the butter of cow's and goat's milk, and also in cocoa-nut and palm-nut oils.

The calcium salt is less soluble than the barium salt; of the latter 0·62 grm. dissolves in 100 grms. of water at 20° C.

The lead salt may be crystallised from alcohol, M.P. 83·5 to 84·5.

Capric acid, $C_{10}H_{20}O_2$, occurs in the form of glycerides in the milk of cows and goats, and in cocoa-nut and palm-nut oils, and as potassium salt in wool washings.

The acid dissolves only in 1,000 times its weight of water and crystallises in needles melting at 31·3° C.

Its alkaline salts dissolve readily in water, its other salts do not; the barium salt is slightly soluble in boiling water, and separates on cooling in crystalline plates; it dissolves, however, readily in alcohol.

Lauric acid, $C_{12}H_{24}O_2$, occurs in traces, as glyceride, in milk, more abundantly in spermaceti and in laurel oil, cocoa-nut oil, and certain other vegetable oils.

Its alkaline salts are soluble, and differ from those of higher acids in being less easily salted out of their solutions; a solution of sodium laurate requires 17 per cent. of sodium chloride to be completely salted out, sodium stearate only 5 per cent., while a dilute solution of sodium caproate is not precipitated by saturation with salt. Use is made of this property in the manufacture of so-called marine soaps.

The other salts are almost insoluble in water; in 1 litre of water at 100, there dissolves 0·55 grm. of calcium salt, 0·70 of barium salt, 0·19 of zinc salt and 0·01 of lead salt. These salts dissolve more readily in alcohol.

Myristic acid, $C_{14}H_{28}O_2$, occurs in traces, as a glyceride, in milk, in lard, in wool-fat, in cod-liver oil, and in large quantities in certain vegetable fats (nutmeg butter and others).

A crystalline potassium salt may be obtained, and also a barium salt that crystallises from alcohol, in which it is somewhat soluble when hot.

Palmitic acid, $C_{16}H_{32}O_2$, occurs combined with glycerol in most animal and vegetable fats, especially in palm oil and Japan wax; also as cetyl ester in spermaceti, as myricyl ester in beeswax, and as ceryl ester in opium wax. Myrtle wax contains palmitic and lauric acids only in the form of glycerides.

100 parts of absolute alcohol dissolve at 19·5° C. 9·3 parts of the acid, boiling alcohol dissolves it easily. It is not very readily dissolved by petroleum ether. It dissolves without change in sulphuric acid and can be recovered by diluting with water.

The alkaline salts dissolve readily in water, less readily in alcohol; 100 parts of 95 per cent. alcohol dissolve 1·14 grm. of potassium salt at 22° C. The silver salt is obtained in crystals by adding alcoholic silver nitrate solution to a solution of ammonium palmitate in alcohol. The calcium, barium and lead salts are very slightly soluble in alcohol; the lead salt is almost insoluble in ether (18 mgrs. in 100 c.c.).

The hydrolytic dissociation of the alkaline salts of fatty acids by water is especially important in connection with the soaps of the higher acids. Alkaline palmitates yield a clear solution in a small volume of hot water, which on cooling sets to a jelly. If the hot clear solution be diluted it becomes clouded, owing to the liberation of free fatty acid and of alkali by hydrolysis. The free fatty acid can be removed by shaking with toluene, and also the amount of alkali set free determined by salting out the unhydrolysed soap and free fatty acid together, dissolving the curd in alcohol, and titrating the solution with alkali. Sodium palmitate diluted with 900 parts of water and then treated in this way was found to contain 4·20 per cent. of sodium instead of the 8·27 per cent. present in $C_{16}H_{31}O_2Na$. In the "acid salt" with the composition $C_{16}H_{31}O_2N_a + C_{16}H_{32}O_2$ there would be 4·31 per cent. of sodium.

This phenomenon has sometimes been referred to as if the monobasic fatty acids formed acid salts. Acid soaps it is true are easily obtained, soaps, that is, containing more or less free fatty acid, but there is no evidence that there is any molecular proportion between the soap and the fatty acid. It is purely accidental that the figures just quoted appear to give colour to such a conception. Krafft and Stern boiled pure sodium palmitate with measured quantities of water, and estimated the percentage of sodium in the salt that separated out on cooling; when 200 c.c. of water was taken for 1 grm. of sodium palmitate, the soap obtained contained 7·01 per cent. Na, with 300 c.c. 6·84 per cent., with 400 c.c. 6·60 per cent., and with 500 c.c. 6·04 per cent. The dissociation is proportional to the dilution. If the partially dissociated soap solution is shaken repeatedly with toluene, very nearly the whole of the palmitic acid can finally be removed. (Krafft and Wiglow, and Lewkowitsch.)

The salts of stearic acid are still more liable to this hydrolytic dissociation than the palmitates. Oleates on the other hand are to a less degree hydrolysed on dilution. Sodium oleate can be dissolved in 10 parts of water, and remains clear on cooling; on diluting this solution twenty times a slight opalescence appears, but it is not perceptibly stronger at four times this dilution.

This hydrolytic dissociation is prevented by the addition of alcohols, ethyl alcohol, 40 per cent., methyl alcohol, amyl alcohol 15 per cent. or glycerol. Hence the rule that in titrating fatty acids with aqueous alkalis, alcohol should be always present at the end of the titration in a concentration of about 50 per cent.

Hydrolytic dissociation is also impeded by excess of alkali, and it is the alkali set free in hydrolytic dissociation that puts a limit to the dissociation and prevents it being complete as might otherwise be expected, from the fact that the liberated fatty acid is insoluble and therefore removed from the sphere of action. With a volatile base like ammonia this limit tends to fall, and ammonia soaps are not stable; they decompose on heating or even on standing.

Stearic acid, $C_{18}H_{36}O_2$, occurs as a glyceride in most solid fats, most abundantly in the vegetable fats, shea butter and cacao butter.

In 100 parts of absolute alcohol at 20° C. 2·5 parts of stearic acid dissolve.

The salts undergo hydrolytic dissociation in water, like the palmitates; even the insoluble barium and calcium salts give up their bases on washing with water.

The calcium and barium salts are almost insoluble in alcohol; the magnesium salt dissolves in hot alcohol and crystallises out on cooling almost entirely.

Lead stearate is almost insoluble in ether, 15 mgrs. dissolving in 100 c.c.

Sodium and potassium stearates dissolve in hot alcohol; on dilution of their solutions with much water acid salts crystallise out.

The copper, lead and silver salts are amorphous.

Arachidic acid, $C_{20}H_{40}O_2$, has been found in traces as glyceride in cow's milk and in the fat of dermoid cysts, more abundantly in arachis or pea-nut oil (5 per cent.) and other vegetable fats

The acid dissolves easily in boiling alcohol, very little in cold much less than stearic acid. It dissolves readily in ether or chloroform, and is also soluble in benzene and petroleum ether.

Its salts are very similar to the stearates; but the copper and silver salts crystallise from alcohol.

Behenic acid, $C_{22}H_{44}O_2$, occurs in oil of ben, used in the East for cosmetics.

It crystallises in needles, which are more soluble in ether (1 in 500 at 16°) than in alcohol (1 in 1000 at 17°).

Lignoceric acid, $C_{24}H_{48}O_2$, occurs as glyceride in arachis oil.

It is very slightly soluble in cold alcohol, readily soluble in ether, benzene and carbon bisulphide.

TABLE OF THE SATURATED FATTY ACIDS.

	Formula.	Mol. Wt.	Solidifying Point.	Melting Point.	Boiling Point.	B.P. of Ethyl Ester.	B.P. of Methyl Ester.	M.P. of Amide.	Sp. Gr.
Formic . .	$HCOOH$	46	—	+8·6	100·8(760)	55	—	—	1·2415 at 0°
Acetic . .	CH_3COOH	60	—	16·5	118	77·5	—	82-83	1·0701 at 0°
Propionic . .	C_2H_5COOH	74	—	−22	140·9	98·8	—	79	1·0133 at 0°
n. Butyric .	C_3H_7COOH	88	−19	−6·5	163·5(760)	119·9(760)	102·3(760)	115	0·9781 at 0°
Isobutyric . .	$CH_3\diagdown\diagup CHCOOH$ CH_3	88	—	−47	155·5(760)	110·1	—	128	0·9651 at 0°
n. Valeric . .	C_4H_9COOH	102	—	−58	186(760)	144·6	127·3	—	0·958 at 0°
Isopropyl acetic	$CH_3\diagdown\diagup CHCH_2COOH$ CH_3	102	−57	−36	173·7(760)	134·3	116·7	127	0·9647 at 0°
Ethyl methyl acetic . .	$CH_3\diagdown\diagup CH.COOH$ C_2H_5	102	—	—	175	—	—	—	0·941 at 21°
Trimethyl acetic	$CH_3\diagdown C.COOH$ $CH_3\diagup$ CH_3	102	—	35	164	—	—	—	0·905 at 50°
n. Caproic .	$C_5H_{11}COOH$	116	—	−20	204·5-205 (760)	165-166	149·6	100	0·945 at 0°

Isobutyl acetic .	CH_3 $>CHCH_2CH_2COOH$ CH_3	116	below −18	−9·5	202-203 (770)	—	—	—	0·925 at 20°
Caprylic . .	$C_7H_{15}COOH$	144	12	16	236-237(761)	207-208	192-194	97-98	0·927 at 0°
Capric . .	$C_9H_{19}COOH$	172	—	31·3	270(760)	243-245	223-224	108	0·930 at 37°
Lauric . .	$C_{11}H_{23}COOH$	200	—	43·5	176(15) 102(0)	[Solid −10] 269	141(15°)	110	0·875 at 43·5°
Myristic . .	$C_{13}H_{27}COOH$	228	—	53·6	200·5(15) 121·5(0)	[Solid 10-11] 102(25)	[M.P. 19] 291(751)	102	0·862 at 53·6°
Palmitic . .	$C_{15}H_{31}COOH$	256	62·6	62·6	340-356(760) 215(15) 138·9(0)	[M.P. 24] 185(10)	[M.P. 28]	106-107	0·853 at 62·6°
Stearic . .	$C_{17}H_{35}COOH$	284	69·3	69·3	ca. 360(760) 232(15) 155(0)	[M.P. 33·7] 200(10)	[M.P. 38]	108·5-109	0·845 at 69·3°
Arachidic . .	$C_{19}H_{39}COOH$	312	—	77	—	[M.P. 50] 284(100)	[M.P. 54]	108	—
Behenic . .	$C_{21}H_{43}COOH$	340	77-79	83-84	306(60)	[M.P. 48]	—	111	—
Lignoceric .	$C_{23}H_{47}COOH$	368	—	80·5	—	[M.P. 55]	[M.P. 56·7]	—	—
Carnaubic . .	$C_{23}H_{47}COOH$	368	69-67	72·5	—	305-310 (15-20)			
Cerotic . .	$C_{25}H_{51}COOH$	396	—	78	—	—	[M.P. 60]	109	—
Melissic . .	$C_{29}H_{59}COOH$	452	—	91	—	[M.P. 73]	[M.P. 74·5]	116	—

Carnaubic acid, $C_{24}H_{48}O_2$, is said to occur combined with higher alcohols in carnaubic wax and in wool-fat.

An acid isomeric with this and the preceding one has been isolated by Dunham from the kidney. It would seem that among these acids to which this formula is given there may be an exception to the general rule that fatty acids that occur in nature have normal chains.

Cerotic acid, $C_{26}H_{52}O_2$, occurs free in beeswax and in carnauba wax; in Chinese wax, opium wax and in wool-fat it occurs combined with ceryl alcohol.

It is extracted from beeswax by boiling alcohol, and crystallises out completely on cooling, in curved needles.

The acid does not dissolve in dilute boiling aqueous soda, but dissolves in boiling alcoholic potash, separating out on cooling. The sodium and potassium salts dissolve in boiling water. The molecular weight has been determined by titration.

Melissic acid, $C_{30}H_{60}O_2$, occurs free in beeswax.

It dissolves readily in hot alcohol, chloroform, carbon bisulphide, or petroleum ether, less readily in ether and methyl alcohol.

The lead salt, insoluble in alcohol or ether, dissolves in boiling chloroform, and crystallises from toluene in needles, melting at 118° to 119° C.

II. The Unsaturated Acids.

These acids contain at some point in the chain one or more pairs of carbon atoms united by a double union. This enables them to combine with halogens and so become saturated, a property which is made use of for determining quantitatively the amount of such unsaturated acids or their glycerides present in a mixture, or the number of double bonds in the molecule of an isolated acid or its ester.

The double union may clearly occur, in the case of the higher members of the series, in a number of different possible situations, and the properties of the acids with a normal chain of a given number of carbon atoms are found to differ with different positions of the double union. For instance the three acids,

$$CH_2 : CH . CH_2 . CH_2 COOH$$
$$CH_3 . CH : CH . CH_2 COOH$$
$$CH_3 . CH_2 . CH : CH COOH$$

are all known, and differ from each other in their properties. The position of the double union in the case of some of the higher members of the series, with only one unsaturated linkage in the chain, has been conclusively determined, by making use of the fact that an unsaturated linkage is liable to become saturated by the entry of hydroxyl groups, and when that is the case further oxidation leads to the chain being broken at this point; the identification of the products of this cleavage indicates the position of the double linkage in the original chain. Instances of the successful application of this procedure will be found in the account given below of the determination of the constitution of oleic and erucic acids.

Some of the cases of isomerism in these acids have in this way been shown to be due to differences in the position of the double union.[1]

Other cases depend on a different fact, namely, that the two un-

[1] Some of the unsaturated acids when fused with potash or even when boiled with an alkali, undergo a change consisting in a transposition of the unsaturated link, *e.g.*, hydrosorbic acid,

$$CH_3 CH_2 CH = CH CH_2 COOH \text{ becomes}$$
$$CH_3 CH_2 CH_2 CH = CH COOH.$$

saturated carbon atoms may have their valencies satisfied by two different arrangements of the same groups, differing from one another in that, in the one the two hydrogen atoms are on the same side, in the other they are on opposite sides, thus—

$$\begin{array}{l} R\text{—}C\text{—}H \\ \quad\| \\ R_1\text{—}C\text{—}H \end{array} \quad \text{and} \quad \begin{array}{l} R\text{—}C\text{—}H \\ \quad\| \\ H\text{—}C\text{—}R_1. \end{array}$$

Instances of this are found in the case of oleic acid and its isomer elaidic acid, and of hypogæic and gaïdic, erucic and brassidic acids (Fittig). That the isomerism in these cases is satisfactorily explained in this way, is borne out by the fact that such isomeric acids, when oxidised and split into two halves in the manner referred to immediately above, yield identical cleavage products. It is remarkable, however, that the measures which bring about the conversion of oleic acid into elaidic acid are without effect in the case of isomers of oleic acid, in which the double union is in a different position from that which it has in oleic, and that in these cases cis-trans-isomerism is not known to occur though theoretically equally possible.

The acids more unsaturated than those of the oleic series occur characteristically in the vegetable drying and semi-drying oils, and also in animal tissues, for instance, in the liver of the cod and many other fish, and also in the liver, heart and kidney of mammalian animals. In the latter they are in part at any rate present in combination with glycerophosphoric acid as phospholipines.

The drying oils are of great commercial and technical importance because of the changes which they undergo when exposed to the air, by which they increase in weight, become saturated by combination with oxygen and are converted into varnishes. Chemical changes of the same kind are undergone by the equally unsaturated fatty acids of animal tissues, though the physical properties of the resulting products are different; cod-liver oil does not "dry" like linseed oil, although it becomes sticky on exposure to air.

The larger the number of unsaturated unions that occur in a fatty acid or its glyceride the more unstable it is, and the more readily it takes up oxygen; the fact that in those tissues of the animal body in which fats are burnt and not merely stored in reserve, the fatty acids found are more unsaturated than those of the glycerides which are found in the adipose tissue, or contained in the food, shows that the former have been got ready for oxidation, while the reserve is kept in the inactive form: the body stores its powder wet for safety, and dries it only when required for use.

The chemistry of the acids more unsaturated than the oleic series has as yet been but little developed; the constitution of none of these acids has yet perhaps been finally determined, and some of the most highly unsaturated acids are known only by their halogen derivatives.

(*a*) *The Oleic Series*, $C_nH_{2n-2}O_2$.

Tiglic acid, $C_5H_8O_4$ occurs as glyceride in croton oil, and is stereoisomeric with angelic acid, the dibromo-derivative of which is converted by sodium amalgam into tiglic acid. By its constitution, as the expanded formula shows, it is an *a*-methyl crotonic acid :—

$$CH_3 - CH = C - COOH$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad CH_3$$

It is soluble in water.

The calcium salt, unlike that of angelic acid, is more soluble in hot than cold water. The barium and silver salts crystallise, the former with 4 molecules of water, which are given off on standing over sulphuric acid.

On fusion with caustic potash it yields acetic and propionic acids (Fittig).

Hypogæic acid and its isomers, $C_{16}H_{30}O_2$.

Hypogæic acid occurs in pea-nut or arachis oil as glyceride, and also in maize oil.

It is easily soluble in cold alcohol, and crystallises in needles. On distillation it decomposes and yields sebacic acid, $C_8H_{16}(COOH)_2$.

Nitrous acid fumes bring about its conversion into *gaïdic acid* by cis-trans-isomeric transformation (Schröder).

Physetoleic acid occurs as the cetyl ester in sperm oil and as the glyceride in Caspian seal oil.

It differs from hypogæic acid in that no stereoisomeric transformation is brought about in it by nitrous acid, and on distillation no sebacic acid is formed.

The barium salt crystallises out of boiling alcohol.

The lead salt is soluble in ether (Ljubarsky).

Palmitoleic acid.—This name has been proposed by Lewkowitsch for an unnamed acid found by Bull in cod-liver oil, and also in herring oil, differing from the isomeric acids as shown in the table of constants. It forms about 6 per cent. of the total acids of cod-liver oil.

Lycopodic acid occurs as glyceride in lycopodium spores. It is fluid at ordinary temperatures. On fusion with potash it yields isobutyric and lauric acids, and when oxidised with potassium permanganate yields dioxypalmitic, isocaproic and oxycapric acid (Langer and Rathje).

Δ^{α} *hypogæic acid* has been synthetically prepared from *a*-iodopalmitic acid by the action of alcoholic potash, which gives *a*-oxypalmitic acid and the corresponding unsaturated acid, $CH_3\ (CH_2)_{12}.CH = CH.COOH$. The calcium salt $(+ 3H_2O)$ is insoluble in water (Ponzio).

Oleic Acid and its isomers, $C_{18}H_{34}O_2$.

Oleic acid occurs as glyceride in most fats and oils, and as a general rule in larger quantities than any other fatty acid.

Pure oleic acid is a colourless, odourless fluid, crystallising at 4° C., which cannot be distilled at atmospheric pressure, but in a current of steam at 250° C. distils unchanged. Insoluble in water, it is far more soluble in alcohol than the corresponding saturated acid, dissolving even in cold diluted alcohol.

The *salts* of oleic acid can mostly be melted without change and are soluble in alcohol, some of them even in ether. The alkaline salts are more soluble than the salts of the higher saturated acids.

Sodium oleate, which can be crystallised from absolute alcohol, dissolves in 10 parts of water at 12° C., in 20·6 parts of alcohol (specific gravity 0·821) at 13°, and in 100 parts of boiling ether. Potassium oleate, which is a jelly, dissolves in 4 parts of cold water, 2·17 parts of alcohol (specific gravity 0·821) at 10°, and 29·1 parts of boiling ether (Chevreul).

Calcium oleate, a powdery solid, dissolves in alcohol and ether. Barium oleate, a crystalline powder, insoluble in water, and very slightly in alcohol or benzene, is said to dissolve in hot benzene, if 5 per cent. or less of 95 per cent. alcohol, or a little water is present, and then on cooling to separate out in crystals almost quantitatively (Farnsteiner).

The lead, iron and copper salts dissolve easily in ether.

The *preparation* of oleic acid from olive oil or lard depends on the solubility of the lead salt in ether. The fat is saponified with alcoholic potash, the acids set free with a mineral acid, separated and heated with lead oxide for several hours at 100°; the lead salts are then extracted with ether. As the salts of the saturated acids are insoluble, the oleate is thus separated from them; the separation is, however, not complete. After removing the ether the residue is treated with hydrochloric acid; the oleic acid is separated, dissolved in ammonia and precipitated with barium chloride; the precipitate is crystallised out of boiling alcohol, decomposed with tartaric acid, and the oleic acid so obtained distilled *in vacuo* (*cf.*, too, *infra* p. 77).

Reactions and Constitution.—Oleic acid cannot be distilled at atmospheric pressure as it decomposes on heating into a number of substances which have thrown no light on its constitution, but among which have been detected acetic, caprylic, capric and sebacic acids, all of which have an even number of carbon atoms.

It is doubtful whether pure oleic acid is appreciably acted on by air at the ordinary temperature. But a stream of air at 120° or a higher temperature converts it into acids insoluble in petroleum ether, and of a higher specific gravity, presumably oxy-acids.

On oxidation with nitric acid all the volatile acids from formic to capric are formed and also several dicarboxylic acids, glutaric, adipic, pimelic and suberic. Permanganate of potassium oxidises oleic acid in alkaline solution, giving at low temperatures mainly a dioxystearic acid, M.P. 134° (Edmed), at a raised temperature pelargonic

and azelaic acids, the mono- and dicarboxylic acids respectively containing nine carbon atoms. The stages in these changes are represented thus:

$$(1)\ CH_3.(CH_2)_7.\overset{H}{\underset{OH}{C}}.\ \overset{H}{\underset{OH}{C}}.(CH_2)_7.COOH$$

dioxystearic acid;

$$(2)\ CH_3.(CH_2)_7.CO.\overset{H}{\underset{OH}{C}}.(CH_2)_7.COOH;$$

this intermediate product has been isolated (Holde and Markusson), and can be converted by chromic acid into

$$(3)\ CH_3.(CH_2).CO.CO.(CH_2)_7.COOH,$$

which is presumably the next product; this then gives rise to

$$(4)\ CH_3.(CH_2)_7.COOH$$

pelargonic acid and $COOH.(CH_2)_7.COOH$, azelaic acid.

This makes it probable that the double bond in oleic acid is in the middle of the chain; for allyl acetic acid, the constitution of which is known in other ways, when thus oxidised, gives formic and succinic acids by breaking up at the point where the double bond occurs:—

$$CH_2:CH.CH_2.CH_2\,COOH \rightarrow HCOOH + COOH.CH_2.CH_2.COOH.$$

There is no change in the position of the double linkage previous to oxidation, and the cleavage products would reveal the true constitution of the original acid were it not previously known.

This probability is increased by the behaviour of oleic acid with ozone. The three atoms of oxygen enter at the unsaturated point in the molecule, as in the case of other unsaturated compounds, with the formation of an ozonide (Molinari and Soncini, Harries and Thieme). From the ozonide the same cleavage-products, pelargonic and azelaic acids, may be obtained.

A third reaction pointing to the same constitution for oleic acid is that described by Baruch. If oleic acid be saturated with bromine and the resulting dibromostearic acid treated with alcoholic potash, stearolic acid is formed, which contains a triple bond, in the place of the double one of oleic acid,

$$C_8H_{17}.C\equiv C.(CH_2)_7.COOH.$$

This substance is converted by concentrated sulphuric acid into ketostearic acid,

$$C_8H_{17}.CO.CH_2.(CH_2)_7.COOH.$$

This compound reacts with hydroxylamine giving a mixture of the two oximes:

$$(1)\ C_8H_{17}.\overset{\overset{N.OH}{\|}}{C}.(CH_2)_8\,COOH \quad \text{and} \quad (2)\ C_8H_{17}.\overset{\overset{HO.N}{\|}}{C}.(CH_2)_8\,COOH,$$

which, with concentrated sulphuric acid, undergo the Beckmann transformation and give rise to

$$(1)\ C_8H_{17}.NH.CO.(CH_2)_8.COOH \quad \text{and} \quad (2)\ C_8H_{17}.CO.NH.(CH_2)_8.COOH.$$

And these substances are broken up by strong hydrochloric acid into

(1)
$\left\{\begin{array}{l} C_8H_{17}NH_2, \text{ octylamine} \\ \text{and} \\ COOH.(CH_2)_8.COOH, \text{ sebacic acid.} \end{array}\right.$

(2)
$\left\{\begin{array}{l} C_8H_{17}\,COOH, \text{ pelargonic acid} \\ \text{and} \\ NH_2\,(CH_2)_8.COOH, \text{ amino-nonylic acid.} \end{array}\right.$

These three changes, pointing to the same position for the double bond in oleic acid, are satisfactory proof that it is in the position which they indicate, namely, exactly in the middle of the chain.

It is true that on fusion with caustic potash oleic acid is broken down into palmitic acid, containing an uninterruptedly saturated chain of sixteen carbon atoms, and acetic and oxalic acids, which shows that under these conditions the weak spot, that is to say the double linkage, is between the α and β carbon atoms. But the migration of a double link in such circumstances is not without parallel (Meyer and Jacobson, and cf. footnote *supra* p. 13).

The evidence that there is as to the course of the biochemical oxidation of oleic acid will be referred to in a later section. There is at any rate no evidence of the formation in animals of either pelargonic or azelaic acid.

Oleic acid dissolves in strong sulphuric acid, being converted into the ester of an oxystearic acid, which oxy-acid, set free by saponification with boiling water, is believed to have the hydroxyl group attached to the ninth or tenth carbon atom (*vide infra* isooleic acid).

Like other unsaturated compounds, oleic acid decolorises bromine water and absorbs two atoms of this or other halogens.

The reduction of oleic acid to stearic acid can be readily effected in the laboratory by heating with hydriodic acid and red phosphorus. For commercial purposes this method is not suitable; others which have been devised have not been entirely successful.

Elaidic acid, $C_{18}H_{34}O_2$. This isomer of oleic acid is formed when nitrous acid fumes are allowed to act on oleic acid for a few minutes. On cooling the fluid sets to a white solid crystalline mass.

The acid melts at 44·5°, and distils *in vacuo* unchanged.

It is easily soluble in warm alcohol.

The solubility of its salts resembles that of the salts of stearic acid rather than oleic acid; the lead salt, for instance, is almost insoluble in ether.

The relation of elaidic acid to oleic acid is shown to be that of a cis-trans-stereoisomer by the following considerations:—

On reduction with hydriodic acid and phosphorus it gives stearic acid.

On oxidation with permanganate in alkaline solution, a different dihydroxystearic acid is obtained, as shown by the lower melting point (99° instead of 132°), the greater solubility in alcohol and the greater readiness with which it is oxidised. But on the other hand, when further oxidised, it yields the same cleavage products, pelargonic and azelaic acids.

With ozone an ozonide is formed which, on heating with water, splits in exactly the same way and yields the same products as the ozonide of oleic acid.

With halogens it yields different addition products from those obtained from oleic acid. But on addition of hydriodic acid to its molecule and then treating the product with alcoholic potash, oleic acid is obtained as well as another acid, isooleic acid.

On fusion with potash it gives palmitic, acetic and oxalic acids ; on treatment with sulphuric acid and then with alcoholic potash it gives the oxystearic acid which is obtained in this way from oleic acid too.

The constitution of elaidic and oleic acids is therefore represented by the formulæ :—

$$CH_3.(CH_2)_7.C.H \qquad \text{and} \qquad H.C.CH_3.(CH_2)_7$$
$$\quad\;\; \| \qquad\qquad\qquad\quad\;\; \|$$
$$H.C.(CH_2)_7.COOH \qquad\quad H.C.(CH_2)_7.COOH.$$

Which of these, however, is which cannot be stated.

Δ^{α} oleic acid has been prepared by Ponzio and Le Sueur by heating *α*-iodo- or *α*-bromostearic acid with alcoholic potash. It crystallises from petroleum ether in needles or plates. M.P. 58° to 59°.

It differs from oleic acid also in giving a lead salt which is not soluble in ether, in being unacted on by nitrous acid, and in not absorbing bromine in the cold.

Its constitution is further indicated by the fact that when oxidised with permanganate in the cold it gives a dioxystearic acid, which is different from that obtained from oleic acid in its melting-point and solubility, and which, on further oxidation on the water-bath, splits up, yielding palmitic acid and not azelaic or pelargonic acids.

This *α-β*-dioxystearic acid melts at 126°, resolidifies at 124-23°, and is very slightly soluble in cold alcohol or ether, and appreciably soluble in water at 100°.

Isooleic acid, $\Delta^{8\text{-}9 \text{ or } 10\text{-}11}$. This isomer of oleic acid has been prepared by Saytzeff and by Ulzer and Klimont.

If oleic acid be treated with sulphuric acid the sulphuric acid ester of an oxy-acid is formed, which, on saponification, yields an oxystearic acid with its hydroxyl group presumably attached to the ninth or tenth carbon atom. On distillation this acid decomposes yielding isooleic and oleic acids, which can be separated, owing to the different solubility of the zinc salts in alcohol or of the acids themselves in petroleum ether. Since these acids differ from one another in these as well as in other respects, the double bond cannot be in the same place, and since they are both formed from the same monohydroxystearic (or iodostearic) acid the double bond in the two acids is most likely to be between one carbon atom, the same in each of them, and one of its neighbours in one of them and the other in the other; if, in oleic acid, it is between the ninth and tenth, then, in isooleic acid, it must be either between the eighth and ninth or between the tenth and eleventh.

Rapic acid, $C_{18}H_{34}O_2$, occurs as glyceride in rape or colza oil, which is expressed from the seeds of the cabbage and other related crucifers.

It differs from oleic acid in not solidifying when acted on by nitrous acid, in not solidifying on cooling, and in the properties of its zinc salts. Its constitution is not known (Reimer and Will).

The following evidence of another oleic acid with the unsaturated link between the sixth and seventh carbon atoms was obtained by Hartley. On oxidising the fatty acids from the liver of the pig with permanganate in alkaline solution at a low temperature, a dioxystearic acid was obtained with the melting point 129·5°, which was constant after recrystallisation from various solvents. This melting point is different from the melting point of dioxystearic acid prepared from olive oil by Edmed, and of the $\Delta^{\alpha\beta}$ acid synthesised by Le Sueur. On oxidising his dioxystearic acid with hot permanganate of potassium Hartley obtained no trace of pelargonic or of azelaic acid, but he found and identified caproic acid, and obtained some indirect evidence for the formation of decamethylene dicarboxylic acid, though this acid was not isolated. Oxalic acid was found at the same time, so probably the dicarboxylic moiety was attacked in the reaction. In the liver of the pig, therefore, an oleic acid occurs, differing from the common one, which is found, as Hartley showed, in the connective tissue of the pig, in having its unsaturated linkage between the sixth and seventh carbon atoms, reckoning from the unoxidised end of the molecule. Reference will be made to the probable significance of this observation later.

Gadoleic acid, $C_{20}H_{38}O_2$, occurs as glyceride in cod-liver oil, in herring oil and in sperm oil (Bull).

On oxidation with permanganate in the cold it yields a dioxy acid, crystallising out of alcohol with the melting point, 127·5° to 128°.

Erucic acid, $C_{22}H_{42}O_2$, $[CH_3(CH_2)_7 . CH : CH(CH_2)_{11} . COOH]$, occurs as glyceride in colza oil, mustard-seed oil or other vegetable oil. The same or an isomeric acid has been found to be present in cod-liver oil by Bull.

In its chemical behaviour it closely resembles oleic acid, but its lead salt is not so soluble in cold ether.

Its barium salt crystallises from alcohol.

It is converted by nitrous acid into the solid brassidic acid. From its dibromide a behenolic acid similar to stearolic acid has been obtained, and from this a keto acid and its oximes, which, similarly to those obtained from oleic acid (*vide supra*, p. 17, *sub* oleic acid), broke up in two different ways, as follows:—

I. $\begin{cases} C_8H_{17}COOH \text{ pelargonic acid} \\ \text{and} \\ NH_2(CH_2)_{12}COOH \text{ 13-amino-brassylic acid,} \end{cases}$ and II. $\begin{cases} C_8H_{17}NH_2 \text{ oktylamine} \\ \text{and} \\ COOH(CH_2)_{12}COOH, \end{cases}$

showing its constitution to be that given above (Baruch).

On fusion with caustic potash it yields arachidic and acetic acids; on reduction with hydriodic acid and phosphorus, behenic acid.

Brassidic acid, $C_{22}H_{42}O_2$. This is a cis-trans isomeride of erucic acid formed from it in the same way as elaidic acid is from oleic acid by nitrous acid fumes. Erucic acid is warmed with dilute nitric acid to 60° to 70° till gas is given off, and then at once cooled.

Isoerucic acid, $C_{22}H_{42}O_2$. This acid has been prepared by methods similar to those which yield isooleic acid (Alexandroff and Saytzeff, Ponzio).

(*b*) *The Linoleic Series*, $C_nH_{2n-4}O_2$.

The acids that have been described in this series have the formula $C_{18}H_{32}O_2$. The acid obtained from linseed oil is the one that has been most studied, but the constitution of even this acid has not yet been determined. An acid, or possibly two acids, of this formula occur in the fat of the pig's liver, and the same is true of cotton-seed oil. And in many vegetable oils a similar acid has been shown to exist.

Two methods have been made use of for preparing the pure acid: (1) The tetrabromide was reduced by Hazura by means of zinc and alcoholic hydrochloric acid; (2) the ethyl ester was prepared by Reformatzky and distilled at 180 mm. at 270°.

Two methods are used for identifying it when present in a mixture of fatty acids:—

(1) The *tetrabromide* is obtained by dissolving the acids in glacial acetic acid and adding bromine slowly: on evaporation the tetrabromide crystallises out. It can be separated from the bromides of other unsaturated acids inasmuch as it is nearly insoluble in petroleum ether, in which the bromide obtained from oleic acid dissolves easily, and because it is easily soluble in alcohol, ether, benzene, chloroform and acetic acid, whereas the hexabromide of linolenic acid dissolves easily only in benzene.

It can be crystallised from alcohol or glacial acetic acid in plates melting at 114°.

(2) The acids are oxidised with permanganate in alkaline solution at a low temperature: linoleic acid is thus converted into tetraoxystearic or sativic acid, 1 grm. of which dissolves in 2 litres of boiling water, and crystallises out on cooling in long needles or prisms which melt at 173°. The acid is insoluble in ether, chloroform and benzene, but dissolves easily in hot alcohol or glacial acetic acid. The dioxystearic acid formed at the same time from oleic acid is more soluble in ether, the hexaoxystearic acid formed from linolenic acid more soluble in water. By heating linoleic acid with hydriodic acid and phosphorus to 200° it may be converted into stearic acid.

The calcium, barium, zinc, copper and lead salts dissolve in ether, the barium salt in benzene and petroleum ether. The lead, zinc and manganese salts dissolve in linseed oil, and then accelerate its oxidation, and are therefore used as "driers". The constitution of the acid has not been determined; but azelaic acid has been found among the products of oxidation by permanganate in alkaline solution and perhaps formic acid (Reformatzky).

Isomers of linoleic acid have been described from other sources: they are identified by the formation of a tetrabromostearic acid, and distinguished from linoleic acid by the melting point of the product so obtained, or of the acid itself from which it is formed.

Linoleic acid or an isomer is assumed to occur in certain animal fats, since the iodine value of the mixed fatty acids yielded by them is higher than that of pure oleic acid.

Hartley has prepared and isolated from the mixed fatty acids, obtained by acting on pigs' livers with strong alkali and alcohol, a tetrabromide and also a tetraoxystearic acid with the melting point 175°. There were indications of the presence of a second acid mixed with this one before it was purified, and it is possible that two acids of the formula $C_{18}H_{32}O_2$ occur in this organ.

The other acids with the formula $C_{18}H_{32}O_2$ which have been described occur each of them in some vegetable oil of local occurrence, and have not been studied as much as linoleic acid. One of these, tairiric acid, is said on oxidation to yield lauric acid $C_{12}H_{24}O_2$ and adipic acid $C_6H_{10}O_4$.

Elaeostearic acid yields valeric and azelaic acids on oxidation with ozone, and consequently contains double bonds probably between the fifth and sixth, and ninth and tenth carbon atoms. It is found in Japanese wood oil, which is described as the best drying oil known (Majima).

(*c*) *Acids of the Series*, $C_nH_{2n-6}O_2$.

The only acids that have been described in this series, as in the last, contain 18 carbon atoms. It is the glycerides of these acids still more than those of the acids of the linoleic series that confer upon the drying oils their peculiar properties.

Linolenic acid was first described by Hazura, then by Hehner and Mitchell, and most recently has been studied by Erdmann, Bedford and Raspe. On brominating the acids obtained from linseed oil, a product containing six atoms of bromine is obtained which is soluble in benzene but in no other ordinary solvent. It melts at 179°. By boiling 50 grms. of this substance with 300 c.c. of 95 per cent. alcohol and 100 grms. of zinc filings Erdmann and Bedford obtained a mixture of zinc salt and ethyl ester free from bromine, and after removing the zinc and saponifying, an acid substance which distilled *in vacuo* at 157° to 158° C., and gave on analysis the figures required by the formula $C_{18}H_{30}O_2$. Two acids were probably present denoted α and β linolenic acids, one derived from the other by, it is supposed, steric change. For from the mixture only 23 per cent. of the original amount of hexabromide could be reformed, the rest absorbing no longer six but only four atoms of bromine and giving a liquid bromide. An attempt to throw light on the constitution of these acids was made by converting the mixture into the ozonides and decomposing these with water. Azelaic acid was obtained in sufficient quantity to account for half the molecule of both modifications, and in addition to this among the oxidation products malonic acid and propionic aldehyde were identified. The final elucidation of the constitution of these acids must, therefore, be left for the future.

Hazura and Hehner and Mitchell also recovered the acid from the hexabromide by reduction with zinc and alcoholic hydrochloric acid, and found that it absorbed not far short of the theoretical amount of iodine (iodine value, 241·8 and 245).

Hazura prepared another derivative by oxidising the acids from linseed oil with permanganate in the cold. Two hexahydroxystearic acids were obtained, linusic and isolinusic acids. These acids are more soluble in water than sativic or tetrahydroxystearic acid, less soluble in alcohol and insoluble in ether. Linusic acid crystallises in rhombic plates or in needles, with the melting point 203° to 205°. Isolinusic acid crystallises in prisms, melting at 173° to 175° C. It has the same elementary composition as linusic acid, but is more soluble in water. But the subject is one that requires further study before these data can be regarded as final.

Linolenic acid can be reduced quantitatively to stearic acid when heated with pumice coated with reduced metallic nickel in a stream of hydrogen at from 170° to 200° C. The amount of hydrogen absorbed corresponds closely with the amount required by theory (Erdmann and Bedford).

(*d*) *Acids of the Series*, $C_nH_{2n-8}O_2$.

Clupanodonic acid is the name given to an acid obtained by Tsujimoto by treating an octobromide isolated from the bromination products of the acids present in Japanese sardine oil. The octobromide was reduced with alcoholic hydrochloric acid and zinc, and the acid so obtained had the iodine value 344 (calculated for $C_{18}H_{28}O_2$ it should be 368). The octobromide is insoluble in ether, blackens at 200°, and then decomposes before melting.

Acids of the formulæ $C_{20}H_{32}O_2$ and $C_{24}H_{40}O_2$ are inferred to be present in herring oil by Bull, on account of the high iodine values obtained.

From the liver of pigs an octobromide has been prepared by Hartley, the analysis of several preparations of which pointed to an acid $C_{20}H_{32}O_2$. The evidence for the occurrence of this acid was made conclusive by the isolation of an acid from the products of permanganate oxidation which, on analysis, proved to be the octohydroxy derivative of this same acid.

Isanic acid is the name given to an acid found in a vegetable oil from the French Congo, to which the formula $C_{14}H_{20}O_2$ is ascribed on account of the analysis of the acid and some of its salts, and the molecular weight of the acid. But the acid absorbed only two atoms of bromine and reduction with hydriodic acid did not give results pointing to its being an aliphatic acid.

Therapic acid is the name given to an acid corresponding to a bromination product obtained by Heyerdahl from the fatty acids of cod-liver oil which, on analysis, appeared to have the composition $C_{17}H_{26}Br_8O_2$.

III. Saturated Oxy Acids, $C_nH_{2n}O_3$.

Lanopalmic acid, $C_{16}H_{32}O_3$, was obtained from wool fat; it forms a potash salt readily soluble in cold alcohol. The acid itself is insoluble in water, but dissolves in it on warming if a little alcohol is added. It crystallises out on cooling. M.p., 87° to 88° C.; solidifying point, 85° to 83° C. (Darmstädter and Lifschütz).

Cocceric acid, $C_{31}H_{62}O_3$, occurs in cochineal wax combined with cocceryl alcohol. The acid is slightly soluble in cold alcohol, ether, benzene, petroleum ether and acetic acid, and crystallises from alcohol. M.p., 92° to 93° C. (Liebermann).

II. UNSATURATED ACIDS: OLEIC SERIES, $C_nH_{2n-2}O_2$.

		Mol. W.	Solidifying Point.	Melting Point.	Boiling Point.	B.P. of Ethyl Ester.	B.P. of Methyl Ester.	Sp. Gr.	M.P. of Dioxy Acid.
Tiglic . .	$C_5H_6O_2$ $CH_3CH:C.COOH$ $\dot{C}H_3$	110	—	64-65	198·5(760)	—	—	0·964(76)	—
Hypogæic .	$C_{16}H_{30}O_2$	254	—	33	230(10)	—	—	—	—
Gaidic . .	$C_{18}H_{30}O_2$	254	—	39	—	—	—	—	—
Δα-Hypogæic	$C_{16}H_{30}O_2$	254	45	49	—	—	—	—	—
Physetoleic .	$C_{18}H_{30}O_2$	254	—	30	—	—	—	—	115
Palmitoleic .	$C_{18}H_{30}O_2$	254	−1·5	—	—	—	185-186(10)	—	125
Lycopodic .	$C_{18}H_{30}O_2$	254	—	Fluid	—	—	—	—	—
Oleic . .	$C_{18}H_{34}O_2$	282	4°	14°	223°(10) 166(0·25)	—	*ca.* 206°(10)	0·898(15°)	131·5-132 Le Sueur
Elaidic . .	$C_{18}H_{34}O_2$	282	—	44-45	154(0)	370° with decomposition	—	—	99-100
Δα-Oleic . .	$C_{18}H_{34}O_2$ $C_{15}H_{31}CH:CHCOOH$	282	56-55	58-59	—	M.p. 25-26	—	—	126 Solid 124-3
Isooleic . .	$C_{18}H_{34}O_2$ $CH(CH_2)_8$ or $_8CH:CH(CH_2)_6$ or $_8COOH$	282	—	44-45	—	—	—	—	77-78 Solid 66-64
Rapic . .	$C_{18}H_{34}O_2$	282	—	Fluid	—	—	—	—	—
Gadoleic . .	$C_{20}H_{38}O_2$	310	—	24-25	—	—	*ca.* 224°(10)	—	127·5-128
Erucic . .	$C_{22}H_{42}O_2$	338	—	33-34	256°(10) 179°(0)	above 360° unchanged	*ca.* 240°(10)	—	132-134
Brassidic . .	$C_{22}H_{42}O_2$	338	56	65	180°(0)	—	—	—	98-99
Isoerucic . .	$C_{22}H_{42}O_2$	338	—	54-56	—	—	—	—	86-88

IV. Unsaturated Oxy Acids, $C_nH_{2n-2}O_3$.

Ricinoleic acid.—The glyceride of this acid is the principal constituent of castor oil. It absorbs two atoms of bromine and gives a monoacetyl derivative with acetic anhydride, and is consequently an oxy oleic acid. On reduction with hydriodic acid it gives stearic acid, and has therefore a normal chain of carbon atoms.

The constitution of the acid has not been definitively settled, but is probably represented by the formula of Goldsobel, which is

$$CH_3(CH_2)_5\,CHOH\,CH_2CH = CH(CH_2)_7\,COOH.$$

This is based on evidence of the same kind as that of Baruch with regard to the constitution of oleic and erucic acids, namely, the formation of a ricinostearolic acid, a keto-oxystearic acid, and its oximes, and the identification of cleavage products of the latter, after undergoing Beckmann's transformation, as γ-dekalactone and azelaic acid.

Further evidence for this formula has been given by Behrend and by Kasansky. But a different formula with which the facts do not so well agree was proposed by Krafft.

On distillation with dilute nitric acid it yields heptoic, oxalic and azelaic acids, which products the formula of Goldsobel might lead one to expect.

On fusion with potash it yields sebacic acid and secondary octylic alcohol.

The pure acid is obtained by repeated crystallisation of its barium salt from alcohol. It melts at 4° to 5°, cannot be distilled unchanged, but at 50 mm. and at 250° C. it is converted into an acid of the composition $C_{18}H_{32}O_2$, which distils over and solidifies on cooling below zero.

It is soluble in all proportions in alcohol and in ether, and insoluble in petroleum ether.

It is optically active, a_D when the acid itself is examined in the polarimeter being + 6·67°.

The calcium salt crystallises in scales from alcohol, and melts at 80°; the barium salt crystallises in plates.

The crystalline lead salt is soluble in ether, and melts at 100°.

The methyl ester boils at 245° at 10 mm.: a_D + 3·8.

The ethyl ester boils at 258° at 13 mm.: a_D + 4·07.

Ricinelaidic acid, stereoisomeric with ricinoleic, is formed when the latter is heated with nitric acid till red fumes form and then quickly cooled. The solid product, pressed free from unchanged substance, is crystallised from alcohol or petroleum ether.

The crystalline needles melt at 52° to 53°.

Ricinic acid, an isomeride of ricinoleic acid, has been obtained by heating the barium salt of the latter (Krafft; *cf.* Walden).

Isoricinoleic acid, another isomeride, is said to be obtained by the action of sulphuric acid. It reacts with phenyl hydrazine and hydroxylamine, and is therefore a keto acid. It dissolves readily in petroleum ether in which medium ricinoleic acid is insoluble.

V. Saturated Dioxy Acids, $C_nH_{2n-1}.(OH)_2.COOH$.

A *dioxystearic acid* occurs in nature only in castor oil so far as is at present known.

This solid acid can be obtained by cooling the acids from castor oil to below 12° and expressing the crystals that form. Stearic acid is removed by dissolving in toluene, and the insoluble dioxy acid crystallised from alcohol.

The melting point, 141° to 143° C., is different from that of the dioxy acids obtained from oleic, elaidic, Δ_α oleic or iso-oleic acids (Juillard).

Lanoceric acid, $C_{30}H_{60}O_4$, is obtained as a potash salt soluble in dilute alcohol on saponifying wool fat with alcoholic potash.

The acid melts at 104° to 105°, but loses a molecule of water, from the two hydroxyl groups, and then melts subsequently at 102°. On boiling with dilute hydrochloric acid it forms a lactone melting at 86° C. (Darmstädter and Lifschütz).

VI. Saturated Dibasic Acids, $C_nH_{2n}(COOH)_2$.

Japanic acid, $C_{22}H_{42}O_4$, is the only dibasic acid occurring in natural fats, small quantities of what is probably the mixed glyceride of this and palmitic acid being present in Japan wax.

It crystallises from alcohol or chloroform in plates which are heavier than water.

On heating to 100° carbon dioxide is given off, and a ketone is formed for which the constitution $C_{10}H_{20}.CO.C_{10}H_{20}$ is given (Geitel and V. der Want).

VII. Cyclic Acids.

Chaulmoogric acid, $C_{18}H_{32}O_2$.—This acid, obtained by Power and Gornall from the vegetable Chaulmoogra oil, has the same composition as linoleic acid; it absorbs, however, only two atoms of bromine, and when reduced by hydriodic acid and phosphorus it yields an acid of the formula $C_{18}H_{34}O_2$. The second unsaturated linkage is supposed therefore to occur in a cyclic formation.

On oxidation with permanganate it yields a dioxy acid, and with excess of the oxidising agent decomposes into lower dibasic acids.

Hydnocarpic acid, $C_{18}H_{32}O_2$, occurring in the same and also in other vegetable oils, is for similar reasons also regarded as a cyclic acid (Power and Barrowcliff).

B. GLYCEROL AND THE GLYCERIDES OF FATTY ACIDS.

1. *Glycerol.*

The triatomic alcohol glycerol occurs in all natural fats and oils, and is formed in the fermentation of sugar by yeast.

At ordinary temperatures it is a viscid fluid and solidifies to a mass of rhombic crystals when cooled much below zero, or at zero if sown with crystals previously obtained. These crystals melt at 20° C. It boils at atmospheric pressure at 290° C. (Mendelejeff), but unless free from salts and impurities decomposes before this temperature is reached. At 0·25 mm. pressure it boils at 143° C. (Fischer and Harries). Its vapour pressure at 118° C. is less than 0·25 mm., at 161° C. 6·5 mm., at 220° C. 101 mm., and at 260° C. 385 mm.

It is miscible with water in all proportions and highly hygroscopic. When mixed with water, contraction and a rise of temperature occur. It cannot be completely freed from water *in vacuo* over sulphuric acid (Struwe). It is readily volatilised with water vapour, so that aqueous solutions heated on a water-bath lose glycerol; in this way it is impossible to obtain solutions of a concentration higher than 70 per cent.

The specific gravity at 15° C. of glycerol and its aqueous solutions is (Gerlach) :—

100	per cent.	glycerol,	1·265	50	per cent.	glycerol,	1·129
90	„	„	1·240	40	„	„	1·102
80	„	„	1·213	30	„	„	1·075
70	„	„	1·185	20	„	„	1·049
60	„	„	1·157	10	„	„	1·024

Glycerol is miscible with alcohol, only slightly soluble in ether (1 in 500), but readily dissolves in a mixture of alcohol and ether. It is insoluble in chloroform, petroleum ether and carbon bisulphide.

It is a good solvent for many salts (Klewer), 100 parts dissolving, *e.g.*, 50 of zinc chloride, 40 of potassium iodide, 30 of copper sulphate, 20 of ammonium chloride or carbonate, 10 of barium chloride or copper acetate, 8 of sodium carbonate, 7·5 of mercuric chloride, and 1 of calcium oleate.

Dehydrating agents are apt to remove two molecules of water and convert it into acrolein, the aldehyde of acrylic acid, $CH_2 = CH \cdot COH$. This occurs especially on heating with acid potassium sulphate.

It is readily oxidised by most oxidising agents. Ozone yields carbonic, formic and propionic acids (Gorup). Hydrogen peroxide, with a trace of ferrous sulphate, yields glyceric aldehyde. Nitric acid gives oxalic, glyceric and other acids (Heintz). With sulphuric acid and manganese dioxide, formic acid and carbonic acid are formed. With dry potassium permanganate it burns explosively (Dvorak); in the presence of excess of alkali this reagent oxidises it quantitatively to oxalic acid and carbonic acid, four molecules of permanganate giving up three of oxygen (Fox and Wanklyn). It reduces many metallic oxides or salts. If heated with silver nitrate on the water-bath till discoloration occurs, on then adding ammonia a mirror is formed. Gold, platinum and mercury salts are reduced in the presence of alkalis, mercury salts simply under the influence of sunlight. Fehling's solution is not reduced by dilute solutions, but strong ones act slowly upo

Halogens partly oxidise, partly give rise to the corresponding hydrines. The lead compound of glycerine is converted by bromine vapour into the mixture of glyceric aldehyde and dioxyacetone, in which the latter predominates, and which is known as glycerose (Fischer and Tafel).

Electrolysis gives rise to formic, acetic, oxalic and glyceric acids and trioxymethylene.

Micro-organisms produce many changes in glycerol. Yeast is said to convert it into propionic acid; the organisms in putrid meat or cheese in the presence of chalk give rise to acetic and propionic, and at the same time to butyric, valeric and caproic acids, and ethyl and higher alcohols.

Bacillus butyricus forms lactic and butyric acid and butyl alcohol (Fitz, Emmerling).

Glycerol forms alcoholates with bases; for instance, the mono- and disodium glycerates, intensely hygroscopic crystalline compounds readily decomposed by water; and the lead glycerate obtained by heating 500 grms. of lead hydroxide with 100 grms. of boiling 85 per cent. glycerine (Fischer and Tafel).

Esters of glycerol may be formed containing one, two or three acid radicals. Those containing one such radical may have it attached to the central carbon atom of the chain, or to one of the terminal ones; and those containing two may have the central or one terminal hydroxyl left unaltered. If there are different acid radicals attached to the same glycerol molecule isomers will arise according to the positions taken up by those acid radicals. The esters formed by the action of the halogen acids, *e.g.*, mono- and dichlorhydrines, and the trichlorhydrine formed from the latter by phosphorus pentachloride, are fluids differing from glycerol in solubility and other properties. Of the esters of nitric acid trinitro-glycerol is familiar for its explosive and pharmacological properties. The ester of phosphoric acid, glycero-phosphoric acid, $CH_2OH.CHOH.CH.OPO_3H_2$, which is found in traces in the urine and blood, is contained in lecithine and other phospholipines, widely distributed through the animal and vegetable kingdoms. It is obtained by the saponification of these compounds with baryta as a soluble barium salt; and it can be synthesised by the action of phosphorus pentoxide or of metaphosphoric acid upon glycerol. Its calcium salt is more soluble in cold than in hot water, and can be obtained in glistening scales by heating a strong solution. The free acid is unstable.

2. *The Glycerides of Fatty Acids.*

The glycerides of fatty acids occur in animals stored in the connective tissue cells of adipose tissue which is found principally in the subcutaneous tissue, the bone marrow, beneath certain parts of the peritoneal, pericardial and pleural serous membranes, and in the interstitial tissue of the voluntary muscles. In this adipose tissue fat the glycerides are for the most part esters of stearic, palmitic and oleic acid. In certain animals the glycerides of other fatty acids occur. In lard the liquid fatty acids have properties (iodine value, *vide infra*) which indicate that 10 per cent. of the acids entering into the composition of this fat are of the linoleic series (Twitchell). In the fat of the horse and the hare there is similar evidence for the presence of glycerides of other acids. It is further remarkable that in certain species the fat of animals kept in captivity differs from that of those living wild. The evidence that points to the presence of linoleic acid esters in the fat of the domesticated pig points to there being more of such esters in the fat of the wild boar. And the same difference is particularly well marked in the rabbit (iodine value of fatty acids from tame rabbit 64, from wild rabbit 101), and the duck (iodine value of fat of tame duck 58, of wild duck 85) (Amthor and Zinck).

On the other hand, this difference does not appear to hold for the cat, and the character of the fat of the chamois and deer living in freedom (iodine value 25 to 30) does not justify generalisations from the facts mentioned.

In the fat of cow's milk the esters of butyric and caproic acids occur in fair amounts (6 to 7 per cent. and 1 to 3 per cent. respectively), and those of the intermediate acids, caprylic, capric, lauric and myristic in traces, as well as that of arachidic acid.

In plants the glycerides of many other acids besides stearic, palmitic and oleic acid occur; for instance, linoleic and linolenic in linseed oil, erucic in colza and rape oil, lauric in laurel oil, myristic in oil of nutmeg, arachidic acid in pea-nut oil, ricinoleic in castor oil, etc.

The fatty acid esters of glycerol that occur in nature contain in all cases three fatty acid radicals and are triglycerides, with the one possible exception of an ester containing two erucic acid groups, a dierucin, which has been separated from a sample of rape oil (Reimer and Will). These triglycerides have frequently been supposed to be each a compound of glycerol with three molecules of one and the same acid, *i.e.*, to be simple triglycerides. But several mixed triglycerides, or compounds of different acids with the same molecule of glycerol, have been separated from natural products. From mutton and beef fat, by means of fractional crystallisation from solutions in acetone or alcohol-ether, a distearopalmitin, a dipalmitostearin and a dipalmito-olein have been separated, and by cooling down ethereal solutions to low temperatures similar mixed glycerides have been obtained from olive oil (Hansen, Kreis and Hafner, Holde and Stange). From butter

the mixed triglyceride, oleopalmitobutyrin, has been obtained by Blyth and Robertson, and from cocoa butter mixed triglycerides of oleic and palmitic with stearic or myristic acids respectively by Klimont and by Hansen. On the other hand, trilaurin has been isolated by distillation of laurel oil in the vacuum of kathode light (Krafft).

The glycerides of the fatty acids up to caprylic acid are liquids heavier than water. Tricaprin melts at 31° C. and the glycerides of higher saturated fatty acids are solids lighter than water.

The melting points of simple glycerides of fatty acids are higher than those of the corresponding acids; those of the mixed glycerides are considerably lower than that of the fatty acid entering into their composition which has the highest melting point (Guth). Pure triglycerides melt or appear to melt at a lower temperature if they have been solidified by rapid and brief cooling than the temperature at which they melt if they have been cooled long enough to become really crystalline. The phenomenon described as the double melting point of triglycerides is probably to be explained similarly. If melted tristearin is rapidly cooled in a capillary tube and then heated it appears to melt at 55° C.; on further heating, to become clouded again and to melt finally at 71·6° C. The rapidly cooled substance is not really crystalline, but in a condition similar to under-cooled water or supersaturated sodium sulphate solution which on mechanical agitation or by the addition of heat, may tend to solidify, thereby liberating heat which contributes to partial reliquefaction and so forth. The determination of the melting point of a glyceride should be undertaken, therefore, only after the specimen has been kept in the solid cooled condition for some hours (Guth, Kreis and Hafner).

The boiling points of glycerides are high, even in the case of those of the lower acids: triacetin 258-59° C., tributyrin 287·8° C. The highest triglyceride that has been boiled without decomposition in a vacuum is trimyristin; tripalmitin decomposes even in the kathode tube before its boiling point is reached (Krafft).

The synthesis of the individual glycerides has been effected in three ways:—

1. By heating glycerol and fatty acids, as was done by Berthelot, in sealed tubes to 200° to 240° C. The monoglyceride so obtained is then heated similarly with more of the acid, and the diglyceride is formed, and by repeating the process with this the triglyceride has been obtained. If a slow stream of dry air is led through a large excess of the acid heated with glycerol, complete esterification with the formation of triglyceride may be attained in one operation (Scheij).

2. By heating the calculated amounts of hydrines with the alkaline salts of the fatty acids. It may be possible in this way to obtain the two different mono- or di-glycerides of a particular acid, starting, *e.g.*, with either α or β mono- or di-chlorhydrine. Several α monoglycerides and α and β diglycerides have been prepared in this way (Guth).

3. By heating the disulphuric acid ester of glycerol with fatty acids dissolved in sulphuric acid (Grün).

The glycerides may be hydrolysed by water alone, in the course of ages at atmospheric temperatures, as is the case with bog butter; in a few hours with superheated steam, and more rapidly in the presence of hydrochloric acid acting as a catalyst. Sulphuric acid acts as a hydrolysing agent more effectively than hydrochloric acid because it helps mechanically to bring the oil into a state of fine division or emulsification; but it acts on the unsaturated oleic acid, giving first an addition product which then is converted into monohydroxy stearic acid, an acid with a high melting point, 81° C. Twitchell's reagent is an aromatic derivative of sulphuric acid combined with fatty acid obtained by dissolving oleic acid in benzene, or naphthalene in oleic acid, and adding strong sulphuric acid. The aromatic sulphonic acid is the catalyst, and it acts similarly to but more rapidly than sulphuric acid, because it is soluble in fat, fatty acids and water alike. The addition of small quantities, 1 to 3 per cent., of lime or magnesia accelerates the action of steam in hydrolysing fats, and if similarly small quantities of the alkalis that give soluble soaps be added the acceleration is greater still.

At the temperature of boiling water, however, even the addition of sufficient alkali to combine with all the fatty acids obtainable from a fat, or even of excess of alkali, does not bring about hydrolysis sufficiently rapidly for laboratory purposes. In the laboratory hydrolysis is carried out usually by means of excess of alcoholic alkali solutions. In this case the greater solubility of fats in alcohol makes the alcoholysis, the formation of ethyl esters of the fatty acids, occur more rapidly than hydrolysis, the formation of the acids themselves, which would occur in water alone. The ethyl esters in the presence of water, however, are themselves hydrolysed, and as the fatty acids are at once converted into soaps by the alkali no equilibrium point is reached in either the alcoholysis of the fat or the hydrolysis of the ethyl esters; the glycerides are consequently completely and rapidly converted into glycerol and soap. The intermediate formation of ethyl esters in the hydrolysis of fats with alcoholic alkalis was demonstrated by Allen, by Kossel and Krüger, and also by Henriques.

The removal of glycerol from its union with fatty acids in glycerides may be effected by alcohols containing as catalyst an acid instead of a base. Methyl or ethyl alcoholic solutions of hydrochloric acid (1 to 2 per cent.) give with fats methyl or ethyl esters of the fatty acids. In the reaction

$$\begin{array}{l} CH_2OOCR \\ CH\ OOCR \\ CH_2OOCR \end{array} + 3CH_3OH \rightleftarrows 3CH_3OOCR + \begin{array}{l} CH_2OH \\ CH\ OH \\ CH_2OH \end{array}$$

the great preponderance of the mass action of the methyl alcohol in which the fat is dissolved over the glycerol originally in combination sets the equilibrium point very much towards the right side of the above equation, and the presence of hydrochloric acid causes this equilibrium point to be rapidly approached.

Enzymes that hydrolyse fats occur in the seeds in which vegetable oils are found, and during germination become active in

preparing the food for the growing embryo. The enzyme in the seeds of the castor oil plant has been used in the technical hydrolysis of fats and oils. Its activity is only fully developed in an acid medium, and is not arrested by acetic acid of less than 12 per cent. concentration. The optimum reaction is $\frac{N}{60}$ to $\frac{N}{100}$ (Connstein, Hoyer and Wartenberg).

Enzymes with a lipolytic action are also well known in animal physiology: the steapsin of the pancreatic juice acts in an alkaline medium, and is easily destroyed by an acid reaction. The lipase contained in an extract of the liver has been specially studied: it hydrolyses not only glyceryl esters but others equally well; but it is sensitive to certain fatty acids, and consequently the esters of all fatty acids are not acted on alike (Kastle).

The reversibility of the action of the enzyme from the pig's liver was shown by Kastle and Loewenhart by the development of the odour of ethyl butyrate when the enzyme was added to butyric acid and alcohol. Fokin was unable to obtain definite evidence of the formation of glycerides from glycerol and fatty acids by the castor oil seed enzyme.

The changes undergone by fats and oils when they become rancid are probably initiated or favoured by enzymes that hydrolyse the glycerides. The free fatty acids are then oxidised by the oxygen of the air in the presence of traces of moisture. But the part played by enzymes and also by the action of light is a subject of some dispute. The changes consist in (1) the appearance of oxy acids and (2) of lower volatile fatty acids or their esters; (3) the hydrolysis of the fat; and (4) the disappearance of the liberated glycerol.

The more saturated acids the fat contains, the less liable is it to become rancid; cacao butter, for instance, is rarely found to undergo this change. The presence in a fat of other substances on which bacteria can grow increases the probability of rancidity; butter, for instance, is particularly prone to the change. But a sterile fat may nevertheless become rancid; and, as Duclaux showed, the growth of bacteria in an impure fat, though it may promote a tendency to rancidity, is not the cause of the change.

C. OTHER ALCOHOLS AND THEIR FATTY ACID ESTERS.

The term wax, which has from common use been applied to substances of very different chemical nature with certain obvious but ill-defined physical properties, has been given a place in chemical terminology which introduces the necessity for restricting its application in some directions and extending it in others. As a scientific term, a wax is defined as a fatty acid ester of some alcohol other than glycerol. Some substances therefore that have been always spoken of as waxes—for instance, myrtle wax or Japan wax—become technically fats ; bees' wax itself is composed only in part of wax. And on the other hand "wool fat" is a mixture of waxes, and liquids such as sperm oil, which contains no glycerides, come to be "liquid waxes". The definition is an arbitrary one, and not very happy, as it would seem to include such substances as ethyl stearate and even ethyl acetate, which is probably more than is intended.

Alcohols that occur in Waxes.

These alcohols are insoluble in water, and are obtained by saponification of the waxes, conversion of the soaps into calcium salts, filtering, drying and extracting the mass with acetone or alcohol. With acetic anhydride the acetic esters are then formed, and these distilled fractionally. For further identification the alcohols may be converted into the corresponding acids by treating with soda lime. By means of sulphuric acid, phosphorus pentoxide or chloride of zinc they can be converted into the corresponding hydrocarbons.

The principal alcohols found in waxes are :—

Cetyl alcohol, $C_{16}H_{34}O$, M.P. 50°, found in spermaceti as ester of palmitic acid, is the principal component of this wax. It occurs too in the secretion of sebaceous glands of ducks and geese (De Jonge), and in dermoid cysts (Ludwig). It crystallises in scales from alcohol. The acetate is slightly soluble in cold alcohol, and crystallises in needles melting at 22° to 23° C. ; it boils at 15 mm. at 200°.

Octodecyl alcohol, $C_{18}H_{38}O$, M.P. 59° C., also occurs as an ester in spermaceti and in the anal glands of geese (Röhmann). It is separated by distillation of the acetate, B.P. 222° to 223° C. at 15 mm. (Krafft).

Eicosyl alcohol, $C_{20}H_{42}O$, has been obtained from the fat of dermoid cysts by Ameseder, and identified by conversion into the corresponding acid, arachidic acid.

Carnaubyl alcohol, $C_{24}H_{50}O$, M.P. 68° to 69° C., is said to occur in wool fat (Lifschutz ; *cf.* Röhmann).

Ceryl alcohol, $C_{26}H_{54}O$, M.P. 79° C., occurs as cerotate in Chinese wax and as palmitate in opium wax, respectively the principal components of these waxes. Also in wool fat, in carnauba wax, and in bees' wax.

Myricyl or melissyl alcohol, $C_{30}H_{62}O$ (or $C_{31}H_{64}O$), M.P. 88° C., occurs as palmitate in bees' wax, which consists mainly of this myricide mixed with cerotic acid. The alcohol also occurs free in this wax together with ceryl alcohol. It is also found free and as the ester of cerotic acid in carnauba wax.

In addition to these, other alcohols of the aliphatic series have been described in various waxes, some of them being unsaturated substances. In sperm oil, for instance, unsaturated alcohols occur.

Furthermore, a glycol, $C_{25}H_{52}O_2$, occurs as an ester in carnauba wax, and another, $C_{30}H_{62}O_2$, cocceryl alcohol, as coccerate in cochineal.

The principal esters occurring in the best-known waxes are given in the following list :—

	M.P.	
Cetyl palmitate . .	53·5°	in spermaceti.
Ceryl palmitate . .	79°	in poppy wax.
Ceryl cerotate . .	82°	in Chinese wax.
Myricyl palmitate .	72°	in bees' wax.
Myricyl cerotate . .	—	in carnauba wax.

As a general rule, these waxes, with the exception of spermaceti, have to be boiled with alcoholic potash much longer than the glyceride in order to be saponified—bees' wax, for instance, for three hours. This is probably due to the low solubility of these solid waxes even in boiling alcohol.

Cholesterol, $C_{27}H_{46}O$ (or $C_{27}H_{44}O$?), a cyclic compound and a secondary alcohol, was first found in gall-stones in 1775 by Conradi, and is found in all animal fats or oils in small quantities—0·1 to 0·5 per cent.—in bile, blood, milk, yolk of egg, and in various animal tissues, notably the medullated sheathes of nerve fibres, the liver, kidney, epidermis, hair and dermoid cysts. Cod-liver oil contains from 0·5 to 2·0 per cent. In the form of a silicate it occurs in birds' feathers (Drechsel).

It crystallises from chloroform in needles containing no water of crystallisation that melt at 148·4° to 150·8° (corr. Bömer). From alcohol, and sometimes from ether, crystals are formed with one molecule of water; they consist of rhombic plates frequently having one re-entering angle. The water of crystallisation is given up slowly over sulphuric acid and rapidly at 100° C. (Bömer).

Its specific gravity is given as between 1·03° and 1·07°. It is laevorotatory, a_D at 15° C., calculated from a solution containing 2 grms. in 100 c.c. of ether, being – 31·1 (Hesse). It is quite insoluble in water: in 100 c.c. absolute alcohol, at 17·5° C., 1·08 grm. will dissolve. It is easily soluble in ether, chloroform, carbon bisulphide or benzene. In 100 c.c. of petroleum ether, boiling under 85° C., its solubility is 0·83 grm. at 19° C. (Bömer). In glacial acetic acid it is only slightly soluble in the cold and when heated dissolves with partial conversion into acetate.

It can be distilled, without decomposition at ordinary pressure if carefully heated, more readily under diminished pressure.

It absorbs two atoms of bromine, and the product dissolves much less readily in a mixture of equal parts of ether and acetic acid than the similar product obtained from sitosterol. If half the theoretical amount of bromine be added to a solution in carbon bisulphide crystals having the composition of one molecule of cholesterol associated with one molecule of dibromide separate and these melt at 112° C. (Cloez).

On heating with soda lime, fatty acids are not formed as they are from aliphatic alcohols (Lewkowitsch).

On prolonged exposure to light and air its solubility diminishes, its M.P. is lowered and the colour reactions become indefinite.

The colour reactions for cholesterol are:—

1. *The Hager-Salkowski reaction:* if a few centigrams of cholesterol, dissolved in 2 c.c. of chloroform, are treated with the same amount of strong sulphuric acid, the chloroform assumes a crimson colour which gradually becomes more purple, while the sulphuric acid shows a green fluorescence. A drop of the purple chloroform solution in a basin turns blue, then green and finally yellow. If the purple solution be diluted with more chloroform it becomes colourless or acquires an intense blue colour, but on shaking again with the sulphuric acid the original colour is restored as the traces of moisture in the chloroform are removed (Salkowski).

2. *Liebermann's reaction.*—Cholesterol dissolved in acetic anhydride gives on adding strong sulphuric acid drop by drop a violet pink colour. The test is sharper, according to Burchard, if to 2 c.c. of a chloroform solution of cholesterol 20 drops of acetic anhydride and 1 drop of strong sulphuric acid are added. The colour changes to blue, by which the reaction is distinguished from a similar reaction given by resin. Wool fat cholesterol reacts with a red colour rather than a violet pink (Lewkowitsch).

3. Cholesterol with concentrated sulphuric acid and a trace of iodine assumes a violet colour that turns blue, green and red.

4. *Schultze's reaction.*—Cholesterol heated with a drop of nitric acid, till the acid is evaporated, leaves a yellow stain that with ammonia turns red.

Cholesterol is obtained (1) from gall-stones by powdering and washing the powder with boiling water and then extracting it repeatedly with hot alcohol, in which the lime salts of bile pigments do not dissolve. The alcohol extract is boiled with alcoholic potash and evaporated down and the cholesterol can then be separated from traces of soap with ether and crystallised from alcohol; (2) from the brain most readily, according to Rosenheim, by mixing the brain in a mortar with plaster of Paris and sand. The mass solidifies and can be easily reduced to a dry powder and then extracted with cold acetone. On evaporation of the acetone almost pure cholesterol separates out. The separation of cholesterol from fats will be dealt with later (p. 88).

The constitution of cholesterol has been and is being investigated in several quarters, but the results so far obtained have not succeeded in clearing the matter up. That cholesterol is an alcohol is evident from the many esters that can be prepared and even exist in nature. That it is a secondary alcohol follows from the formation of the corresponding ketone, cholestenone, $C_{27}H_{44}O$, on oxidation with copper oxide at 280° to 300° C. (Diels and Abderhalden), or on treating the dibromide dissolved in benzene with potassium permanganate and sulphuric acid and then removing the bromine with zinc and acetic acid after it has done its work in protecting the doubly-bound carbon atoms from oxidation. In this way Windaus obtained the ketone in a yield of 60 per cent. crystallised from methyl alcohol. Cholestenone melts at 81° to 82° C. That it contains a doubly-bound carbon atom follows from the fact that it adds on (1) the atoms of hydrochloric acid, when this gas is passed through a solution of cholesterol in alcohol and ether: the chlorocholestanol, of which 50 per cent. is obtained, melts at 154° C. (Mauthner and Suida); (2) two atoms of bromine when dissolved in carbon bisulphide and treated with a solution of bromine in the same solvent, or when cholesterol in 10 per cent. solution in ether is treated with half its weight of bromine in 10 per cent. solution in glacial acetic acid (Windaus); the dibromide melts with decomposition at 102° to 103° C.; (3) and that with Hübl's solution it gives the theoretical iodine value (*vide infra*, pp. 69 and 88).

This double union is thought by Windaus to be at the end of a side chain and not in the ring.

Both cholesterol and cholestenone can be converted into a saturated alcohol cholestanol ($C_{27}H_{48}O$) on reduction with sodium and boiling alcohol (Neuberg and Rauchwerger). This saturated alcohol is not the same as coprosterol which is formed in the intestine from cholesterol.

Cholesterol is thought to contain a saturated cyclic system; according to Windaus and Stern this is a reduced retene formation, methylisopropyl phenanthrene; it exhibits therefore relationship to the terpenes. Neuberg and Rauchwerger point out that certain colour reactions given by cholesterol are also given by a retene derivative, abietic acid. Many other derivatives of cholesterol have been prepared and described which contribute less immediate illumination on its constitution.

Coprosterol, $C_{27}H_{48}O$, a reduced cholesterol formed in the intestine from the cholesterol of the food, was described by Bondzyncki and Humnicki. It is soluble in cold absolute alcohol and dissolves easily in ether, chloroform or benzene. It crystallises in fine needles melting at 95° to 96° C.: $a_D = + 24°$. It gives most of the colour reactions of cholesterol, though with differences; the Salkowski reaction at first gives only a yellow colour that gradually turns brown and finally red. With Liebermann's reaction the colour is blue from the first, turning green later.

Coprosterol does not absorb bromine and can be separated from cholesterol by bromination of the latter and extraction with petroleum ether.

On the fate of the cholesterol contained in the bile, after it reaches the intestine, *cf.* Dorée and Gardner.

Hippocoprosterol, $C_{27}H_{54}O$.—A still further reduced cholesterol is found in the fæces of herbivora (horse, ox, rabbit, sheep: Dorée and Gardner), but appears to be contained in grass and to be excreted unchanged, and therefore not a product of the reduction processes in the intestine acting on cholesterol. It crystallises out of boiling alcohol in fine needles, melting at 74° to 75° C., which are dextrorotatory. It gives Liebermann's reaction (*cf.*, too, Gittelmacher, Wilenko).

Isocholesterol occurs together with cholesterol in wool fat. It is said to occur also in vernix caseosa (Ruppel). It is obtained by treating wool fat with cold alcohol which dissolves out cholesterol. The insoluble remainder is heated with alcoholic potash in a sealed tube or an autoclave at 100° C., the alcohol evaporated, the residue mixed with water and shaken with ether. The residue, after evaporating the ether, is heated with four times its weight of benzoic acid to 200° C., or with benzoyl chloride for a few minutes to 160°. The benzoates, after washing with potash, are taken up in ether, the ether evaporated and the residue after boiling out with alcohol is crystallised from ether. The benzoate of cholesterol crystallises in plates, M.P. 150° to 151° C., that of isocholesterol in minute needles, M.P. 194° to 195° C., and they can be separated mechanically. The benzoate when saponified yields the alcohol.

Isocholesterol is less soluble than cholesterol in cold alcohol, dissolves in hot alcohol, and separates as a jelly on cooling. It crystallises from ether, in which it dissolves readily, in needles, M.P. 137° to 138° C.

It is dextrorotatory, $a_D = + 60°$ (Schultze).

It behaves differently from cholesterol when tested by the colour reactions. (1) In chloroform solution with strong sulphuric acid the solution slowly turns brown, not crimson (Salkowski's reaction); (2) with acetic anhydride and strong sulphuric acid it gives a yellow coloration which turns reddish yellow with a green fluorescence; (3) the yellow stain left when isocholesterol is heated to dryness in nitric acid turns yellowish red with ammonia.

Isocholesterol undergoes a change when kept for years exposed to light resulting in a lowering of the melting point.

Phytosterol. In oils derived from vegetable sources a substance occurs that was taken for cholesterol till certain differences were noted by which it was distinguished and given the name phytosterol. On analysis phytosterol appeared to be an isomeride of cholesterol, but differed from it in its melting point and its crystalline form, and it could be separated and distinguished too after acetylation.

The melting point of phytosterol from different sources was, however, given very various values, from 135° to 144° C., and similar irregularities were observed in the physical properties of its derivatives. These facts seemed to point to the presence of more than one substance in the preparations.

The crystalline form of phytosterol was given as that of thin flattened needles, pointed at either end, which under the polarising microscope appear dark when the plane is parallel to or at right angles to the long axis of the crystal. With cholesterol the extinction planes run approximately parallel to the diagonals.

The most certain means of distinguishing phytosterol from cholesterol and therefore for detecting adulteration of animal with vegetable oil is to acetylate the unsaponifiable cholesterol or phytosterol or mixture of the two by boiling the crystals with a little acetic anhydride for one minute in a basin covered with a watch-glass and then evaporating the excess on a water-bath. The residue is crystallised from a small quantity of absolute alcohol, and the crystals, after repeating the crystallisation several times if a mixture is suspected, will give the melting point 125° C. if it be pure phytosterol acetate, 114·5° C. if pure cholesterol acetate. The admixture of phytosterol is indicated by a melting point higher than this latter figure after several recrystallisations even when, according to Bömer, as little as 1 per cent. of a vegetable oil is present in the original oil from which the alcohols were obtained.

The facts cited above, however, suggest that there should be some doubt about the chemical individuality of phytosterol. And as a matter of fact in the phytosterol of Calabar beans Windaus showed that two substances were present, sitosterol and stigmasterol (Windaus and Hauth).

Sitosterol, $C_{27}H_{44}O + H_2O$, was first obtained from the oil of wheat and rye. Burian prepared it by extracting the lime salts of the fatty acids with acetone, and recrystallising the extract from methyl alcohol. The crystals obtained from dilute alcohol contain one molecule of water and are plates; from ether anhydrous needles are obtained. The melting point is 137·5° C. (uncorr.) (Burian). A preparation from maize oil gave the same melting point; the crystals sometimes had re-entrant angles like cholesterol, more often bifid ends compared to swallow tails. It is laevorotatory, $a_D - 26·7$.

It gives the colour reactions of cholesterol.

It forms a dibromide when treated in solution in carbon bisulphide with bromine. This dibromide is, unlike that of cholesterol, soluble in a mixture of ether and glacial acetic acid. The acetate is obtained by heating with acetic anhydride in the form of white scales, M.P. 127° C. This can be brominated and the product crystallised from dilute alcohol.

Stigmasterol, $C_{30}H_{48}O$ or $C_{30}H_{50}O$, was obtained by Windaus by treating the crude phytosterol of the oil of Calabar bean with acetic anhydride and then the acetylated product in ethereal solution with bromine dissolved in acetic acid. An insoluble compound containing four atoms of bromine was thrown down. This crystallised from a solution in hot chloroform by the addition of alcohol forms four or six sided plates, melting at 211° to 212° C. It is very slightly soluble in ether or acetone, easily soluble in chloroform. The bromine can be removed with sodium amalgam or zinc dust and acetic acid and the resulting acetate saponified; the alcohol so obtained crystallises in needles or elongated thin plates with pointed ends, which contain one molecule of water of crystallisation, melt at 170° C., and give Salkowski's and Liebermann's reactions.

Dissolved in chloroform stigmasterol exhibits the rotatory power, $a_D = -45·0°$.

Stigmasterol has been found to be present also in the "phytosterol" of rape oil and cacao butter, but not in that of linseed oil. These facts, therefore (Windaus and Hauth), suggest an explanation of the discrepancies concerning the physical properties of "phytosterol" obtained from different sources.

Esters of cholesterol and isocholesterol occur in certain fats of animal origin, but the isolation and identification of them has seldom been successfully carried out.

An alcohol extract of blood serum was evaporated and extracted with ether; the residue obtained on evaporating the ether was heated with ethyl acetate and a solution of cholesterol esters was obtained, from which lecithine separated out as the solution cooled. From this solution on concentration the esters crystallised out, and by subsequent crystallisation from alcohol the oleate was separated from palmitate and stearate (Hürthle).

A silicic acid ester of cholesterol was found by Drechsel and Winogradoff in the feathers of birds.

The esters of cholesterol and isocholesterol which are believed to exist in wool fat have not been isolated. Nor were the esters of octadecyl alcohol in the extract of the anal glands of geese and ducks successfully isolated.

D. PHOSPHOLIPINES.

Compounds of fatty acids containing phosphorus and nitrogen.

A common class name for these substances is desirable, but does not exist. Thudichum gave the name "phosphatides" to a number of such substances that he prepared from the brain, because it expressed or was intended to express the peculiar relationship in which he supposed the phosphorus stood to the other constituents of the molecule, a relationship for which there is no evidence, which does not either accord with the facts, and which has not been accepted by any one else. Nevertheless, the term phosphatides has been very generally adopted by those who have worked with these substances, and the indefinite nature of the term has led to its application frequently to substances that are not compounds of fatty acids at all. And it is indeed impossible to say what kind of substances should be regarded as phosphatides and what not, unless it implies merely the presence of phosphorus and oxygen.

The term lipoid was first used by Overton perfectly justifiably, without any chemical connotation, for substances resembling in their solubilities the fats. Such a term was for his purposes necessary; and it was used by him appropriately without regard to chemical relationships. But to adopt it deliberately as a class-name in a classification based on chemical constitution is impossible. For it may be and commonly is used to include anything soluble in ether, or even alcohol, and nothing but confusion can result if it is pretended to mean anything that can be chemically defined.

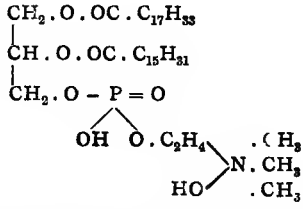
It is common for difficulties to arise over the nomenclature of new groups of bodies the chemical character of which is not exactly known. For ill-defined substances names must be indefinite and provisional. But as soon as it is possible to define the common features of a class of substances names that correspond to this definition, and are, so to speak, in themselves an abbreviated definition, should be substituted for the inappropriate, indefinite and provisional earlier ones. And though it is natural to hesitate before the risk of causing confusion by adopting a personal nomenclature, there are cases in which existing terminology cries aloud for extermination.

In these pages, therefore, for the compounds of fatty acids containing phosphorus and nitrogen the term phospholipine will for short be used, the termination implying a basic character and the presence of nitrogen, the rest of the word implying the presence of fatty acid radicals and phosphorus. For those compounds of fatty acids that contain nitrogen but no phosphorus for similar reasons the term lipines will be employed.

Lecithine.

Lecithine was the name given to a substance or substances obtained by Gobley in 1847 from the yolk of egg, from nerve tissue, the ova of fish and other materials; he showed the presence of fatty acids, phosphorus and nitrogen in his preparations, and that the phosphorus was present as glycerophosphoric acid.

Lecithine was further studied by Hoppe Seyler and his pupils some twenty years later, and the constitution of this phospholipine was defined as a result of analyses and the identification of the products of its hydrolytic cleavage. These latter were three in number—fatty acids, glycerophosphoric acid and choline. The preparations analysed agreed with the supposition that these cleavage products were combined in the original lecithine molecule as follows: In glycerophosphoric acid one of the alcoholic hydroxyl groups of glycerol is the means by which an ester is formed with phosphoric acid. In this ester therefore two alcoholic hydroxyl groups are left free, and two acid hydroxyl groups also are free. In lecithine the two alcoholic hydroxyl groups of glycerophosphoric acid form ester unions with two fatty acid molecules, while one of the acid hydroxyl groups is free, and the second is united with the alcoholic base, choline. Choline, oxyethyltrimethyl ammonium hydrate, could unite with an acid hydroxyl group either by means of its alcoholic hydroxyl or by means of the hydroxyl attached to the nitrogen, its basic hydroxyl group. And reasons were given by Strecker and Gilson for the belief that it was the former kind of union that existed in lecithine. The constitution of this substance was therefore represented by the structural formula—



in which other fatty acid radicals might take the place of the oleyl and palmityl radicals inserted in the form here given.

The solubility of lecithine in solvents for fats and the difficulty of isolating it quantitatively, or even at all, led to the practice of estimating the phosphorus content of the substances extracted by solvents for fats, and from this by the use of the above formula calculating the amount of lecithine present. The errors involved by this practice were exposed by the work of Thudichum on the brain many years ago, and more recently it has become apparent that this practice is not justified in any tissue. There are other phospholipines besides lecithine, and even the "pure" preparations of lecithine, the analysis of which led to the traditional formula, were probably not single substances, or at any rate had undergone some change during the protracted operations to which they were submitted in preparing them for analysis, and there is no guarantee that the whole of any preparation had been changed uniformly, or that a single modification was the result.

The complete chemical characterisation and identification of these phospholipines is left for the future, and even the best results that have been attained have much that is not final about them. It would, therefore, be a mistake to describe systematically the different forms of these bodies that have been given names, as if they were securely established chemical entities. They will be referred to severally in a later section where the methods in use for examining compounds of fatty acids are described. Here, however, a short account of their general properties may be in place and more particularly of those constituents of their molecules, which when these go to pieces can be isolated and identified as definite and characteristic chemical substances.

Lecithine was prepared by Diakonow from the yolk of egg by shaking it with ether repeatedly till the ether took up very little colouring matter. What was left of the yolk was then treated with alcohol at 50° to 60° C., quickly filtered and concentrated, the syrupy residue washed with ether and then dissolved in a small quantity of alcohol and the solution cooled to – 15° C.; the lecithine separated out and was then dried *in vacuo*.

Much of the lecithine was contained in the ether used for removing pigment and fat; this was recovered by Gilson, who distilled off the ether, took up the residue in petroleum ether and extracted from this solution the lecithine by shaking it with 75 per cent. alcohol. The alcoholic solution was left some time to get quite clear, then decolorised with charcoal, and concentrated at 50° to 60° C. The syrupy residue was taken up in ether, filtered and evaporated, and this residue dissolved in alcohol and allowed to separate from solution in the cold.

The lecithine obtained in this way is a yellow, viscid, waxy substance, soluble in chloroform, carbon bisulphide, benzene or oils, less soluble in ether and petroleum ether, soluble in alcohol, especially when warmed, and comparatively insoluble in ethyl or methyl acetate or acetone unless heated. The addition of acetone to an ethereal or chloroform solution causes precipitation of the lecithine.

In contact with water it shows the remarkable "myelin forms". A smear of lecithine on a glass slide under a drop of water, if observed through the microscope, can be seen to protrude rounded projections which gradually elongate and become more and more abundant and intricate. If this process be allowed to take place in a test tube or other vessel that can be shaken, agitation causes the water to become turbid through the dispersion of the delicate microscopic myelin protrusions, and in course of time a uniform emulsion of the lecithine in water is obtained, a suspension of fine swollen particles that constitutes a colloidal solution that can be filtered without change.

Such a colloidal solution of lecithine is not coagulated by heat, nor precipitated by salts of monobasic or tribasic metals, but is precipitated by those of dibasic metals. Salts of calcium in the concentration of 0·01 M, of strontium in that of 0·025 M, and of barium in that of 0·033 M, cause a precipitate that gradually coheres to a gelatinous mass. Acids also cause precipitation if sufficiently dissociated; thus sulphuric acid 0·005 M is effective, but carbonic acid has no effect (Koch).

Lecithine gives Raspail's or Pettenkofer's reaction with strong sulphuric acid and sugar, on account of the unsaturated fatty acids it contains; the purple product dissolved in acetic acid shows a band between D and E, and in chloroform one between C and D, and another broad region of absorption between D and G (Thudichum, p. 121).

A combination of lecithine with hydrochloric acid has been obtained and analysed by Thudichum, who mentions also combinations with potassium and silver. Compounds with various salts are formed; with sodium chloride a compound soluble in ether, precipitated by alcohol and insoluble even in hot acetone (Bing); with mercuric chloride a compound soluble in ether, but insoluble in alcohol; an alcoholic solution is precipitated by platinum chloride ($PtCl_4$ or H_2PtCl_6), and by cadmium chloride (Strecker, 1869). The platinum double salt is soluble in ether, the cadmium salt is not. The formation of the platinum combination is accompanied by some decomposition of the lecithine (Gilson), and the action of cadmium chloride also is not simply to give an addition product (*cf. infra*, p. 95 *seq.*).

Lecithine is optically active (Ulpiani) which is accounted for by the unsymmetrical construction of the glycerophosphoric acid that is obtained from it (Willstätter and Lüdecke, Power and Tutin).

If alcoholic solutions of lecithine and of glucose be evaporated down together, the residue soluble in ether contains glucose as well as lecithine. The proportion of glucose and lecithine varies according to the conditions; and if the ethereal solution be precipitated with alcohol three times as much sugar is contained in the precipitate as in the substance left in solution, and the amount of fatty acids obtainable by saponifying the precipitate is considerably less than it should be if the substance precipitated were a simple combination of lecithine and glucose. The evaporation of lecithine and glucose together in alcoholic solution results not in the formation of a chemical compound of the two unaltered substances but in changes in the unstable lecithine which are at present not fully understood (Baskoff). A lecithine glucose containing 85 per cent. of glucose was analysed by Mayer, but the figures do not correspond to a combination of lecithine and glucose (*cf. infra*, *sub* jecorine, p. 102); for the substance analysed contained absolutely more nitrogen than phosphorus and less carbon than is contained in glucose.

Lecithine was supposed by Hoppe Seyler to be combined with protein in the vitellin of egg yolk. Lecithine can, though with great difficulty, be removed by alcohol from vitellin, but that the protein and lecithine are chemically combined in constant proportion is very doubtful (Hammarsten). In the same way it is difficult to remove lecithine by extraction with alcohol from an artificial mixture of protein and lecithine (Liebermann). Beyond this fact there is no evidence whatever that lecithine and proteins form definite chemical combinations of constant composition.

Combinations of lecithine with alkaloids are noted by Bing. The reactions between lecithine and cobra venom, and lecithine and the toxin of bees are from the chemical point of view entirely obscure.

Lecithine is very readily partially saponified by alkalis: the complete saponification of lecithine, however, is effected by alcoholic potash less readily than that of fat, according to Erlandsen's observation, that even after boiling for eight hours with alcoholic potash the fatty acids obtained from lecithine were not absolutely free from phosphorus.

Lecithine is also saponified by acids, *e.g.*, 10 per cent. sulphuric acid: in this case much of the glycerophosphoric acid will be saponified too.

If a solution of baryta in water or in methyl alcohol be used for the saponification, the fatty acids are obtained as insoluble barium soaps, and the other cleavage products, choline and the glycerophosphate of barium, which is soluble in water, are obtained in solution.

Lecithine is saponified by the lipase of the pancreas (Mayer).

The fatty acids obtained from lecithine by Hoppe Seyler and his pupils were believed to be the same as those that occur in animal fats generally, stearic, palmitic and oleic. But it has been frequently observed in recent years that the iodine value of the acids obtained from lecithine is too high for them to be only these. Henriques and Hansen noted iodine values for the mixed acids from egg lecithine of about 100, and for the fluid acids separated from these of 154. Hartley and Kennaway and Leathes found considerably higher values for the mixed acids from the "lecithine" of the liver of pigs and of the human liver.

The lecithine of the heart muscle was found by Erlandsen to yield fatty acids with the iodine value 110, and of the mean composition on analysis $C_{18}H_{32}O_2$, that of linoleic acid. The formula for the lecithine that he analysed, calculated from his analytical results, allows $C_{35}H_{64}O_4$ for two molecules of fatty acids, or more probably twice this for four molecules obtained from two lecithines containing different fatty acids. What the acids actually were it is impossible, of course, to say; but even supposing the acids had not been altered during the various operations through which they had been carried, they must have contained some of the unstable acids more unsaturated than the oleic series, and probably this is one of the reasons why lecithine and some of the other phospholipines that resemble lecithine in this respect, or even outdo it, are so exceedingly unstable and so difficult to work with. Thus a preparation of cuorine analysed by Erlandsen was found after being kept for some time in dry air to contain thirty atoms of oxygen in the molecule instead of twenty-one. Even the half-hour's heating with alcoholic potash necessary for saponifying a preparation of phospholipines from the liver could be shown to have caused a lowering of the iodine value of the fatty acids from 137, the value calculated from the iodine value of the unsaponified substance, to 121, the observed value of the acids themselves [Leathes]. Some of the substances described by Thudichum—oxy-kephaline, oxykephaloidine and peroxy-kephaline—represent almost certainly different stages in the spontaneous change undergone by the substances originally present in the brain on exposure to the treatment to which he subjected them, in which he was not guided by knowledge of the frailty of the fatty acids entering into their composition.

The glycerophosphoric acid is obtained, when lecithine or a similar substance is saponified with baryta, as a barium salt soluble in water, insoluble in absolute alcohol. The aqueous solution on concentration over sulphuric acid gives a crystalline residue, laminæ, that give the acrolein reaction for glycerol, and on incineration barium phosphate. The aqueous solution of the barium salt may be precipitated with lead acetate, and the lead salt, which is almost insoluble even in hot water, may be tested for glycerol and phosphoric acid, and identified by determination of the latter or of the amount of lead that it contains.

The glycerophosphoric acid cannot be obtained quantitatively when lecithine is saponified (Strecker, Erlandsen), so that the estimation of lecithine by the amount of glycerophosphoric acid obtained from it is not practicable.

The calcium salt of glycerophosphoric acid, like the barium salt, crystallises in platelets, and both salts are also more soluble in cold than in hot water.

Choline, oxyethyltrimethyl ammonium hydrate, is obtained when lecithine has been saponified with baryta, after filtering off the barium soaps and removing excess of baryta with carbonic acid by evaporating the filtrate down to dryness and taking up what will dissolve in absolute alcohol: choline dissolves, glycerophosphate of barium does not. This alcoholic solution treated with an alcoholic solution of platinum chloride gives a light yellow precipitate which is washed with alcohol, dissolved in water, and left to crystallise on slow concentration over sulphuric acid. The orange-coloured crystals form superposed clusters of plates which under the microscope can be seen to be hexagonal. They contain 31·64 per cent. of platinum.

Choline can be detected when very small amounts are obtainable by evaporating a solution of some salt, preferably the double platinic chloride, on a slide and adding a drop of a solution of 2 grms. of iodine and 6 grms. of potassium iodide in 100 cc. of water; dark brown prisms or plates will appear, and then as the fluid evaporates disappear to reappear again on adding another drop of the iodine solution (Rosenheim, Bocarius).

The yield of choline is also not quantitative. Erlandsen from his lecithine obtained only 42 per cent. of the theoretical amount; others have been unable to get as much as this. McLean, who has specially studied this, finds that from Riedel's "lecithol" saponified with baryta in methyl alcohol or water his yield was 78 per cent., but with a preparation of lecithine from the heart the yield was only 40 per cent. He concludes that some other base was present as well.

Some of the other closely related phospholipines give on hydrolysis other bases—cuorine, for instance, a base the platinum double salt of which contained 37·3 per cent. of platinum, and in other respects differed from choline; and kephaline, obtained by Koch from the brain (*vide infra*, p. 97), a base which he takes for a monomethyl choline. But none of these bases have yet been fully studied.

Galactolipines and Lipines.

Compounds of fatty acids containing nitrogen but no phosphorus.

Several substances have been isolated from animal material which contain nitrogen but not phosphorus, and the results of elementary analyses clearly show that they are derivatives of fatty acids though generally somewhat anomalous acids. Some of these are compounded with the carbohydrate galactose, galactolipines in the scheme of nomenclature adopted in these pages, the cerebrines or cerebrosides as they are usually termed; others, containing no carbohydrate radical, according to the same scheme may for the sake of uniformity be classed as the lipines. These include the amidolipotides of Thudichum and certain other substances for the existence of which there is also some evidence.

Galactolipines.

Of the galactolipines the one that has been most completely studied is the *cerebrone* of Thierfelder. This is probably the same substance as was described by Gamgee under the name pseudocerebrine and probably the same too as Thudichum's phrenosine, though Thudichum himself says that his preparations always contained about 1 per cent. less carbon than the formula that he ascribes to this substance requires.

Thierfelder obtains cerebrone thus: human brains reduced to a pulp are dehydrated with acetone and extracted by shaking with ether; the ether after standing for a time at 0° C. deposits a precipitate that is added to the rest of the brain pulp; this is now extracted with 85 per cent. alcohol at 45° so long as the alcohol gives any precipitate on cooling to 0° C. These precipitates are dissolved in five parts of a mixture of methyl alcohol and chloroform (1 : 3); the precipitate formed on cooling is now recrystallised from a mixture of methyl alcohol and chloroform in the proportion of 4 : 1. Traces of phosphorus are removed by means of a solution of zinc hydrate in methyl alcohol containing ammonia and ammonium acetate, which is added to the hot solution in methyl alcohol and chloroform (9 : 1): the mixture filtered hot gives a precipitate on cooling which is recrystallised from methyl alcohol containing 10 per cent. of chloroform. This is cerebrone. It is, as the above account of its preparation shows, a component of the material known formerly as protagone, and the fact that it is obtained from this merely by the discriminating action of certain solvents is one proof among many that protagone is not a single substance. When cerebrone is heated with 10 per cent. sulphuric acid in methyl alcohol for three hours on the water-bath and cooled to 0°, an acid, partly in the form of methyl ester, is found in the precipitate, $C_{25}H_{50}O_3$, to which the name cerebronic acid is given, and from the filtrate the sulphate of a basic substance, sphingosine $C_{19}H_{39}NO_2$, separates on concentration, and from the fluid diluted with water and boiled, galactose is formed out of methyl galactoside.

Thudichum's phrenosine gave similar cleavage products, sphingosine, galactose and an acid, neurostearic acid, an isomer of stearic acid. For sphingosine Thudichum gives the formula $C_{17}H_{35}NO_2$, making it a lower homologue of Thierfelder's base.

The bulk of the molecule both of sphingosine and of cerebronic acid is clearly closely related to the fatty acids though their precise constitution has not been determined, and consequently their relation to the other compounds of fatty acids that occur in animal chemistry is obscure.

"Cerebrins" have been described too by Kossel and by Koch, and another "cerebroside" by Thudichum, kerasine. Koch's cerebrin is possibly cerebrone: the other bodies have been less completely studied than cerebrone.

Another galactolipine was obtained by entirely different methods by Bethe, who treated the brains of horses with copper chloride and potash on the lines of the method of Schmiedeberg for preparing nucleic acids; the barium salt of an acid was finally obtained which was soluble in benzene, chloroform, hot water or hot alcohol, but insoluble in ether and could be resolved into its constituent parts, galactose, an aminocerebrinic acid of the same elementary composition as Thierfelder's sphingosine mentioned above, and an acid free from nitrogen, cerebrinic acid, $C_{19}H_{36}O_2$. This product, therefore, is very similarly constituted to cerebrone, but no further work has been done upon it.

Lipines.

A class of substances obtained by Thudichum, called by him amidolipotides, and another called cerebrin acids, are an indication of the existence of these substances in the brain. Whether they are originally present as such in the brain substance, or whether they are derivatives of more complex substances is open to question perhaps. Certain compounds, such as sphingosine, entering into the composition of cerebrone or phrenosine, and also Bethe's aminocerebrinic acid, both referred to above, appear to be similar substances that do occur in combination with other groups in larger molecules, and possibly the same holds good for Bethe's phrenine.

The only one of these lipines that has been met with and described by more than one worker is *sphingosine*. This cleavage product of the galactolipines has already been mentioned in the last section. The base was first described by Thudichum as obtainable from phrenosine when heated to 130° C. with 2 per cent. sulphuric acid for several days. It is soluble in alcohol and in ether; its salts are not. It is insoluble in water though its salts are somewhat soluble. It gives Raspail's reaction (Pettenkofer's) with sugar and sulphuric acid. Its elementary composition points to the formula $C_{17}H_{35}NO_2$. Its salts crystallise, the hydrochloride in needles. The nitrate gives no precipitate with silver, but is precipitated by mercurous and mercuric nitrate, by lead, copper and cobalt nitrates and by platinic chloride, quantitatively by a saturated aqueous solution of picric acid (Thudichum, p. 187 *seq.*). Under certain conditions

phrenosine is split so as to give the base psychosine, $C_{23}H_{45}NO_7$, which can be further decomposed so as to yield sphingosine and galactose (Thudichum, p. 196). Psychosine crystallises from alcohol. Its hydrochloride is easily soluble in water, differing in this respect from the salt of sphingosine.

Sphingosine or psychosine is also obtained on hydrolysis of kerasine, the other galactolipine described by Thudichum, and also from the phospholipine sphingomyeline (*vide infra*, pp. 95 and 97). (Thudichum, p. 171, but *cf.* pp. 111, 112). This substance, which contains two atoms of nitrogen to one of phosphorus, would then appear to have one nitrogen atom in the form of choline or neurine and the other in the form of the lipine sphingosine.

Thierfelder found a base split off on hydrolysis of cerebrone to which at first he ascribed the same formula as Thudichum to his sphingosine. Subsequently, however, he substituted the homologous formula $C_{19}H_{39}NO_2$; but mixed with this in the cleavage products of cerebrone is another base which was not completely isolated or investigated.

Rosenheim and Tebb found sphingosine among the products of the action of baryta and also of hydrochloric acid upon "protagone".

Aminocerebrinic acid obtained by Bethe from the brain of the horse is given the formula $C_{19}H_{37}NO_2$, which brings it into very close relationship with both Thudichum's and Thierfelder's sphingosine. When heated with potash it gives off ammonia, and the nitrogen-free residue dissolves readily in ether, and gives the same melting point (84° C.) as Thierfelder's neurostearic acid.

Two "amidolipotides," krinosine and bregenine, were described by Thudichum. *Krinosine* is extracted by boiling ether from crude preparations of the galactolipine kerasine, and separates in a felt of fine threads on cooling. It does not give Raspail's or Pettenkofer's reaction with sulphuric acid and sugar; on analysis it appears to have the formula $C_{88}H_{70}NO_5$. *Bregenine* is soluble in benzene and in ether in the cold and in warm alcohol. It does not give Raspail's reaction. On analysis it gives figures with which the formula $C_{40}H_{81}NO_5$ agrees.

Cerebrinic acid is the name given by Thudichum to another lipine which is precipitated by lead acetate and ammonia, and so freed from the galactolipines. The precipitate dissolves in benzene. Its elementary composition, still more its constitution, is uncertain.

Another lipine was obtained by Bethe having the composition indicated by the formula $C_{56}H_{111}NO_8$. This is clearly a substance closely related to some of the above, and Bethe points out the resemblance, particularly to krinosine.

None of these lipines have, however, up to the present time been sufficiently studied for it to be clear exactly what their relationship to other compounds of fatty acids is.

There are indications in Thudichum's work also of the occurrence of compounds of fatty acids, which contain sulphur, of possible thiolipines. But though this reminds one of the question of the sulphur in jecorine, no definite product was isolated even by Thudichum.

CHAPTER II.

EXTRACTION AND ESTIMATION OF FAT.

THE fat or oil in animal or vegetable tissues is obtained for commercial purposes by methods which are seldom available in physiological investigations. The material is heated by itself or with water, and the melted fat poured or skimmed off; or, especially in the case of vegetable matter, seeds, nuts, etc., the oil is expressed by means of hydraulic presses. In the physiological examination of animal tissues these methods cannot generally be employed. The fat of adipose tissue can, of course, readily be obtained by melting it and straining it off from the connective tissue, vessels, etc. But when the connective tissues from those parts in which fat is usually deposited in the largest amount happen to contain but little fat, or when an exact determination of the amount of fat is necessary, recourse must be had to extraction by solvents or some other method.

The extraction of fat from the body or the organs of an animal with solvents cannot be effected quantitatively without change in the nature of the compounds in which the fatty acids are present. The tissue must either be dried and powdered, in which case drying at high temperatures in the air results in the partial oxidation of the more unstable unsaturated acids, as well probably as in other changes in the nature of some of the more complex fatty substances; or else the drying may be effected by means of alcohol, in which case the subsequent extraction with ether can hardly lead to exact quantitative results.

The extraction of fat, therefore, in a condition as little altered as possible, must be dealt with as a problem distinct from that of the estimation of the amount of fat in animal tissues.

A. THE EXTRACTION OF FAT.

The most efficient solvents for animal fats, *e.g.*, ether, chloroform, benzene, petroleum ether, are immiscible with water. Consequently it is necessary that the material to be extracted should be dried before it is treated with these liquids, if the extraction is to be at all complete, and the dried material must then be powdered so as to give the solvent the best chance of reaching the soluble fats enclosed in the great mass of insoluble substances composing the tissue.

The methods used for drying the material to be extracted are :—

1. Drying in air or neutral gas.
2. Drying by means of alcohol.
3. Drying by means of salts, anhydrous sodium or calcium sulphate.

1. *Drying in Air or Neutral Gas.*

The readiness with which the unsaturated acids undergo oxidation when heated in the air makes it necessary to avoid the procedure that suggests itself most readily and has been actually most used, that is to say, to *avoid heating the material in open vessels on water-baths or in ovens*, if it is desired to obtain the fatty substances unaltered and as they exist in the animal body. The unsaturated acids and the combinations in which they are found in animals are partially converted when so treated into substances insoluble in petroleum, with difficulty, if at all, soluble in ether, and easily soluble only in alcohol.

The lower the temperature, of course, the less change will be effected by drying in the air, and therefore it may be sometimes practicable to make use of the plan, resorted to by Erlandsen in the study of the fatty substances of the heart and muscles, of *drying in a current of air at a low temperature.* An electric fan directs a current of air over glass plates on which the finely minced tissue is spread out in a thin film. The air can be slightly warmed by means of Bunsen burners placed near the fan. In some laboratories an enclosed gallery several feet long and a square foot or more in section is constructed of sheet-iron through which filtered air is drawn. By heating this near the inlet the temperature of the air can be raised to the body temperature if desired. One side of this gallery is on hinges, so that the dishes or plates can be introduced into the interior or examined from time to time without removal. It is, however, generally necessary to keep the surface constantly renewed, so that for many purposes such a contrivance presents no advantage; and without it, it is possible to reduce the weight of large amounts of, for instance, liver pulp 50 per cent. by drying before a fan for six hours with air warmed to about 30° to 35° C. It is, of course, not possible to dry the material completely in this way; nor is it in all cases possible to obtain it in a condition in which it can be powdered, especially if it contains much fat. But the greater part of the remaining water can be removed by the use of alcohol, and then the material may be reduced to a condition of finer subdivision by appropriate means.

Where small quantities of material only have to be dealt with it may be advantageously dried under diminished pressure at a raised temperature in an apparatus that can be constructed as follows: A framework carrying a tier of trays, on which dishes can be placed, is made of a size and shape adapted to a stout bell jar that can be exhausted over a metal plate. Through the metal plate two holes are drilled, through which by an air-tight joint the two ends of a long loop of flexible metal tube pass. One end of this tube serves for the admission of steam or water at any desired temperature which escapes by the other end; above the plate the loop of metal tube is coiled in a series of flat horizontal coils which are supported by the metal frame, and on each of which one of the flat dishes containing the material to be dried is supported, the whole being covered by the bell jar. A third opening through the metal plate carries a tube for the admission of a slow stream of dried air or carbonic acid, which escapes by a tube through the tubulure at the top of the bell jar which is connected with a pump. If a rubber washer with a luting of soft soap be used between the bell jar and metal plate, the apparatus can be kept at a low pressure even when steam is passed through the coils. The glass does not get hot owing to the poor conduction of heat through the vacuum, and there is but little danger of a suitable bell jar not standing the pressure.

2. *Drying by Alcohol.*

Minced or pulped animal organs may be treated with an equal volume of alcohol, and after some hours the fluid strained and pressed off. This first alcoholic liquor removes a large part of the water and very little of the fatty substances. Thus in one case 840 grms. of liver pulp containing fatty substances, estimated as fatty acids soluble in petroleum ether after saponification, amounting to 8·8 per cent., was treated with 900 c.c. of spirit and the expressed fluid contained in all 1·13 grms. of fatty substances estimated in the same way, or 0·13 per cent. of the pulp, or less than 1·5 per cent. of the total fat that it contained. The fatty substances that are thus removed will clearly, from their solubility in such aqueous alcohol, be phospholipines not simple glycerides, and the iodine value of the fatty acids they contain is high.

The undissolved mass is then treated repeatedly with fresh quantities of alcohol, and the expressed alcoholic liquors mixed, filtered and evaporated *in vacuo* at a low temperature with a fine, slow stream of carbonic acid or some inert gas passing through a capillary tube beneath the surface. When the alcohol and water have boiled off, the residue can be taken up in ether.

After the tissue has been in this way dried with alcohol it can be reduced to powder and extracted with ether. The ether extracts are freed from solvent at a low temperature and pressure, and the residue again taken up in ether can be mixed with the ethereal solution of the alcoholic extracts.

After four successive extractions with alcohol followed by two with ether there remained unextracted in the liver pulp, in the experiment referred to above, 2·3 grms. or 3·1 per cent. of the total fatty substance of the liver.

The first portion of alcohol with which the tissue is treated necessarily contains after expression much water. And on evaporation *in vacuo* this gives trouble from frothing. It is convenient, therefore, not to mix it with the subsequent alcoholic liquors, but either to disregard it or evaporate it separately.

If the pulped tissue is first dried in a current of air at 30° to 35° C., the treatment with alcohol can be subsequently carried out to complete the removal of water, and in this case the alcohol extracts will contain no more water than can be conveniently removed on evaporation *in vacuo* without frothing, and a difficulty and source of loss is avoided.

It would probably be wise where it is desired to obtain the most sensitive fatty substances from a tissue with the least possible alteration, to use for drying the tissue alcohol that has been recently freed from dissolved oxygen by boiling: for the coefficient of absorption of alcohol for oxygen is about twenty times as great as that of water for this gas. And in any case the drying under alcohol should be carried out in closed vessels.

If the risk of change from contact with air at the temperature of a hot water-bath is in any case immaterial, and the drying of the tissue is to be effected in the old way in an open vessel at a high temperature, then too the practice of adding alcohol to the tissue in small portions at a time, and as each portion evaporates of rubbing the material down with a pestle in the evaporating basin, greatly facilitates, as is well known, the final pulverisation of the dried substance.

3. *Drying by means of Anhydrous Salts.*

Anhydrous sodium sulphate rubbed into an intimate mixture with moist tissues is converted into the hydrated salt at the expense of the water of the tissue, and in this way a dry, easily pulverisable mass can be obtained after a few hours (Pinkus, Schryver). In the case of blood this method is particularly convenient; 100 grms. of the anhydrous sodium salt during complete conversion into the hydrated crystals takes up about 125 grms. of water.

The mass of material obtained in this way is, however, inconveniently large, and it would probably be better to inspissate the blood or tissue pulp in a current of air at about 35° C. before treating with the salt. It is possible in this way to reduce the mass of dried mixture of hydrated salt and powdered substance for extraction about 40 per cent.

Calcium sulphate has been similarly used (*cf.* Rosenheim); but since 100 grms. of the anhydrous calcium salt takes up only about 25 grms. of water the bulk of material for extraction becomes very great. If the removal of water is effected entirely by this means, at least 3 grms. of plaster of Paris must be taken for 1 grm. of most animal tissues, whereas 0·6 grm. of anhydrous sodium sulphate should be sufficient for this same amount of the same tissue.

When the material is dried the pulverisation of small quantities in a mortar by hand presents no great difficulty; but where large

quantities have to be dealt with some mechanical assistance becomes necessary if a really fine state of subdivision is aimed at. In this case the appliances that are serviceable are different in the case of different tissues. Muscular organs can be ground in some of the mills used for grinding coffee or pepper; but the liver, as well as any material that contains at all large amounts of fat, is more readily dealt with in a mechanical mortar or a "drug mill" constructed on the principle of the mills used for grinding mortar, and in this case the grinding may be done under alcohol.

But if the original mincing of the fresh tissue was efficiently carried out, and it be recognised that we are at present concerned with the extraction and not the quantitative estimation of fat in animal tissues, then it is at least questionable whether the labour of pulverising the dried material to the finest state possible will be repaid in the additional yield of material. With the liver, at any rate, this is the case. Finely minced liver easily gives up to alcohol and ether, without any laborious powdering of the dried material, all but 2 or 3 per cent. of its compounds of fatty acids. With some other tissues the powdering of the dried substance may become more necessary, though here too no doubt care and attention will be more profitably expended upon the mincing of the fresh substance.

A form of mincer that gives very good results is known commercially as the "Russwin" mincer, while many of the mincers in common use in laboratories are comparatively inefficient and render the labour of subsequent grinding much more necessary. Material that has been dried with sodium sulphate can be easily pulverised, if it was originally finely minced, even without the use of mechanical mortars.

A ball mill worked by a motor is recommended by Völtz.

B. THE ESTIMATION OF FAT IN ANIMAL TISSUES.

The fat found in those parts of the body where fat is stored, for the use of cells elsewhere rather than in the storage cells themselves, is almost entirely composed of simple glycerol esters of fatty acids easily soluble in ether and slightly in alcohol. The fat found in the cells of the liver and in those of the organs in which fat is oxidised for the liberation of energy is largely composed of complex phospholipines, which present solubilities different from those of the simple fats.

In most of the estimations of fat that have been carried out in physiological investigations, particularly in those of earlier date, the nature of the fat in the product weighed has not received proper attention. Whatever was soluble in ether has commonly been estimated as fat whether it was entirely composed of the simple glycerides or whether 50 per cent. or more of it was composed of phospholipines. The criterion in determining the value of a method of fat estimation has been the gross yield of substances soluble in ether. More recently criticism has been concerned with the "purity" of the fat estimated. The same method has been commended on the one ground, and condemned on the other. The method, for instance, introduced by Rosenfeld, which has in recent years very commonly been taken as the standard method of fat estimation, was so regarded because it gave higher yields of substances soluble in ether than other methods. It has been subjected to adverse criticism because the extract weighed contains nitrogen and phosphorus and a low percentage of fatty acids. This is unreasonable. Lecithine and other phospholipines are soluble in ether, contain nitrogen and phosphorus, and compared with simple glycerides a low percentage of fatty acids, 70 per cent. in the case of lecithine, as little as 40 per cent. in the case of some others. A method which aims at giving the gross weight of substances present that are soluble in ether must necessarily give with most animal tissues a product resembling that obtained by Rosenfeld's method in that it is not "pure fat".

A more pertinent criticism of such a method arises out of the considerations discussed above in the section on the methods of fat extraction, and would question the possibility of obtaining, by any extraction method that should be called quantitative, the compounds of fat that occur in animal tissues, in an unaltered state. And even if this were possible, the lack of homogeneity in the substance weighed would make such an estimation irrational, and of little value even for comparative purposes, since the composition of the extracts obtained from different parts of the body, or from the same part in different animals, may be entirely different.

Many investigators have used methods of estimating fat which are alike in that the substance estimated is not the sum of the more or less unaltered compounds of fatty acids soluble in ether occurring in the tissues, but the fatty acids that can be set free from these combinations. These higher fatty acids are not only the chemical

component common to all these compounds, but they constitute from the functional, or physiological point of view the nucleus in the molecule of each of them. Physiologically considered the higher fatty acids are the common centre of interest in simple glycerides and complex phospholipines alike. It is they that confer upon both these groups their significance in the transformations of energy which constitute the life of an organism. And till it is possible to determine separately the amount of each of the different compounds of higher fatty acids that occur in an animal tissue, there is more to be said for a method which gives the total amount of these acids present in the tissue than for one that merely gives the weight of ether extract, in which these acids may figure in any proportion from 40 to 95 per cent.

These general preliminary considerations show that there can at present be no such thing as a theoretically perfect method of estimating fat, but that the methods which have been employed may be grouped together under two heads:—

1. Those in which the attempt is made to obtain all the substances soluble in ether which may be present in the tissue; these may for convenience be referred to as the *extraction methods.*

2. Those in which the higher fatty acids are set free by saponification from the various combinations in which they may occur, separated as far as possible from other products of the action of the alkali used in saponification and weighed as fatty acids; these methods may be referred to similarly as *saponification methods.*

1. *Extraction Methods.*

The inherent defects of the methods hitherto practised, which are based on dissolving out the fats from an animal tissue, have been indicated above. They may be briefly summarised here:—

1. The tissue must be dried, and if heating in the air is resorted to for effecting this the unsaturated acids are partially oxidised, and their weight on the one hand thereby increased, their solubility in fat solvents on the other hand altered. This will make most difference in the case of those tissues in which the compounds of unsaturated acids are most abundant, *e.g.*, the liver, unless unusually fatty, lean muscle substance, normal kidneys in most animals, thymus, spleen, pancreas, etc.

2. The extract weighed is of very varying composition according to the organ examined, or its condition at the time of examination. The extract from one liver may contain over 90 per cent. of fatty acids, while that obtained in the same way from the majority of livers contains no more than 60 per cent.

The methods that have been proposed and used for the estimation of fat in dried and powdered animal tissues have differed from one another (1) in the solvent used, and (2) in the devices resorted to for liberating the fats entangled in, or possibly combined with, the dried protein substances.

It was shown by Pflüger that extraction with ether, however prolonged, failed to yield the full amount of fat present. From a comparative study of the extracting power of different solvents Kumagawa and Suto found that the solvent that gave the largest yield of substances soluble in ether was alcohol used near its boiling point in a continuous extraction apparatus. Such an apparatus may be made as they recommend, so that the tube containing the material to be extracted, which is fitted with a syphon tube similar to that of a soxhlet extractor, is held in the wide neck of the flask in which the alcohol or other solvent is boiled, or it may be extemporised by hanging from the cork of a wide-neck flask, through which the tube of a condenser passes, a paper thimble containing the substance to be extracted. The mouth of the thimble is plugged with cotton wool, and is so placed as to catch the drops of condensed solvent and to hang in the vapour of the boiling fluid. When the extraction is complete the solvent is completely evaporated off, the residue dried, taken up in ether and then filtered.

Rosenfeld's method, which has in recent years been much used, consists in boiling the thimble containing the dry powder in a beaker containing alcohol, covered with a watch-glass and heated on a water-bath, then extracting in a soxhlet with chloroform for four to six hours, repeating the treatment with alcohol and the extraction with chloroform, evaporating alcohol and chloroform to dryness separately and taking up the residues in ether, mixing, filtering, evaporating and drying.

The extract that is weighed as fat is dark, and contains probably always much that is in no sense of the word fat. The extract from the liver or heart contains more nitrogen on saponification, and yields a lower percentage of fatty acids than if it were all lecithine.

The liberation of fat from the dried protein in which it is entangled, or with which it has sometimes been on insufficient evidence supposed to be chemically combined, has been attempted by digestion with pepsin and hydrochloric acid after a preliminary extraction of the powder with ether. The digested solution is then finally filtered, the filter dried and extracted, and the filtrate shaken repeatedly with ether (Dormeyer). The combined ethereal solutions yield 10 per cent. more extract than can be obtained by simple continuous extraction with ether in a month, but, according to Rosenfeld, 30 per cent. less than can be obtained by his method.

It is sufficiently clear that the uncertainty as to the composition of the extract obtained by either of these methods is not compensated for by any special convenience or expedition in the procedures involved.

Other extraction methods of fat estimation have been proposed which have no advantage over these and have been less generally used. The most recent, that of Glikin, is an attempt to obtain a purer extract, partly at any rate freed from lecithine. The dry powdered material is extracted with petroleum ether, and the petroleum solution purified with acetone, in which lecithine is insoluble or but slightly soluble. Glikin's paper contains a comparison of the results obtained by his method with those obtained by other methods, of which it is largely a criticism.

2. *Saponification Methods.*

Liebermann described a method for determining the amount of higher fatty acids soluble in petroleum ether that can be set free from the various combinations in which they occur by the action of strong potash. His directions are to boil 5 grms. of the dry powdered substance with 30 c.c. of potash (specific gravity 1·54 = 65 per cent.) for half an hour in a flask of special form. The neck of the flask is 19·5 cm. long and 3·6 cm. in diameter. The total height of the flask is 25 cm., and its capacity up to the middle of the neck is 290 c.c., the diameter at the widest part being 7·5 cm. The alkaline solution is after this treated with 30 c.c. of alcohol and heated for ten minutes more. To the cooled solution 100 c.c. of 20 per cent. sulphuric acid is added gradually, while the flask and its contents are continually cooled. Into the acid mixture 50 c.c. of petroleum ether are then introduced, the flask corked with a rubber stopper and shaken thirty times at intervals of a minute for ten seconds each time. The cork is then removed and saturated sodium chloride solution run in till the level of the subjacent aqueous fluid is up to a mark on the lower part of the neck indicating the capacity of the vessel up to this level of 240 c.c. The flask is again stoppered and shaken a few times, and left till a complete separation of clear petroleum has taken place. Of this 20 c.c. is then removed with a pipette into a weighed flask, treated with 40 c.c. of 96 per cent. alcohol, and titrated with 0·1 N alcoholic potash and phenolphthalein (1 c.c. 1 per cent. alcoholic solution). The fluid is then evaporated on the water-bath, dried in a boiling-water oven for an hour, and the residue weighed. From the weight of the soap is then deducted 10 mgrms. for the phenolphthalein and the weight of potassium contained in the amount of potash used for titration, less the corresponding weight of glycerol; or if x be the number of c.c. used, the deduction amounts altogether to $10 + x \times 2{\cdot}55$ (*i.e.*, 3·91 − 1·36) mgrms. This gives the weight of glyceride corresponding to the amount of soap obtained from 20 c.c. of the petroleum solution or two-fifths of the dried powder originally taken (Liebermann and Szekely). This calculation rests on the assumption that the only compounds of fatty acid present in the material originally taken were triglycerides.

The minute directions given above from Liebermann's original account may safely be departed from in certain particulars, and in many cases that are likely to arise with advantage. In the first place the estimation can be, and in the light of what is now known with regard to the effect of drying animal fats should be, carried out on the fresh tissue. Then frequently it may not be possible to take the amount of fresh tissue that would contain 5 grms. of dry solids, and yet accurate results can be obtained with proper care.

In certain respects too the method may be improved. The use of rubber corks should be dispensed with, and flasks with ground stoppers substituted, because rubber takes up petroleum ether and so alters the volume. The drying of soaps in open vessels as carried out by Liebermann is to be avoided, and the fact that the petroleum will take up cholesterol as well as fatty acids should be taken into account, and steps taken for the removal of this.

The modifications that in the experience of recent investigations have been found desirable lead to the account of the method practised by myself which follows :—

From 10 to 20 grms. of the fresh moist material is weighed out to the second place of decimals in a basin. The amount to be taken should be determined roughly by the amount of fatty acids likely to be obtained from it ; this amount should be from 0·3 to 0·7 grm. Of a liver or heart, therefore, yielding the usual amount of fatty acids, about 3 per cent. of its fresh weight, from 10 to 20 grms. is to be desired ; but when this amount is not available an estimation can still be carried out, with 5 grms. or perhaps even less. Into the basin is then measured roughly a corresponding amount, 10 to 20 c.c., of a solution of caustic potash made by dissolving potash sticks in two-thirds of their weight of water, and the basin is heated under a watch-glass on the water-bath till the tissue is disintegrated. If it is stirred occasionally with a glass rod, and the bath be boiling, this takes half an hour. Then 10 to 20 c.c. of spirit are stirred in, the watch-glass removed, and the heating continued with occasional stirring for an hour. Sometimes, if drops of unsaponified fat are visible, more alcohol may be necessary. The soap is then washed with hot water into a flask with a neck 8 inches long and 1 inch internal diameter and a bulb having the capacity 100 to 200 c.c. which should vary according to the amount of fluid that will be obtained. The flask and contents are cooled under the tap, and 15 to 30 c.c. of 40 per cent. sulphuric acid are cautiously added, with constant cooling, so as to avoid frothing over. When this has been done it is convenient to heat the flask in the water-bath for a few minutes to drive off gas. Then, after again cooling, either 25 or 50 c.c. of petroleum ether, exactly measured, are added, according to the amount of fatty acids expected to be present, the object being to obtain a solution of between 0·5 and 1·5 per cent. The volume of fluid in the flask is, by the addition of water or saturated brine, so adjusted that the column of petroleum lies entirely in the neck of the flask and in the lower part of it.

The stopper moistened with glycerine is then put in, and the flask shaken for an hour (certainly not less than prescribed by Liebermann). Steps must be taken to ensure the stopper not being driven out by the pressure in the flask, which, however, will be slight if the flask was heated as directed after adding the acid and then sufficiently cooled. The flask is then left for an hour or more till a layer of clear petroleum has separated, when an aliquot part is removed with a pipette, such an amount as will contain not less than 0·15 grm., preferably 0·2 or more, and run into a weighed round-bottom flask with a short, wide neck and a capacity of 150 to 200 c.c. From this flask the petroleum is distilled off in a water-bath while a current of CO_2 is kept passing through. When the distillation is completed the receiver is connected with the water-pump, the CO_2 stream reduced, and the apparatus exhausted with the flask immersed in the bath for half an hour. The flask is then removed to a desiccator and, when cold, weighed.

The crude fatty acids so obtained will contain cholesterol and probably other unsaponifiable matter. And since this is present in varying amounts, and may in some cases form nearer 20 than 10 per cent. of the crude fatty acids, it is clearly desirable that it should be removed, and its amount determined. This will only be possible with any degree of accuracy when somewhat larger quantities of the crude fatty acids are obtainable, 1·0 grm. or more. In that case the mixture may be dissolved in hot alcohol and made alkaline (after being titrated if desired) with potash, and the solution transferred to a separating funnel so as to contain about equal amounts of alcohol and water. It is then shaken with petroleum ether, the lower layer removed and shaken with fresh petroleum, and the upper layer shaken with fresh alkaline 50 per cent. alcohol. The combined petroleum solutions contain the unsaponifiable substances, which can be weighed after removing the petroleum.

Such a method as that here described is at almost every point a compromise. But in order to make the estimation of fat a practicable operation compromises are necessary, and they are necessary too for the approximation to accuracy. Complications that are theoretically desirable may defeat themselves not only by making an estimation impracticable in the time available, but also by introducing fresh sources of error with every fresh operation.

Excellent modifications of the saponification method have been described by Kumagawa and Suto, and by Mottram. But there is at present a lack of finality about some of the details of technique, and for that reason the simplest method is preferable.

In preparing animal organs for estimation of fat care must, of course, be taken to remove adipose connective tissue. In the case of the liver the large vessels and the gall-bladder should be removed; in that of the heart the auricles and even the right ventricle must as a rule be sacrificed entirely; the basal portion of the ventricle, the valves, and the entire epicardial surface must be cut away, especially along the course of the vessels; also the endocardial surface should be similarly cleared of all that is not pure myocardial substance; the kidney should be split longitudinally by three parallel cuts exposing the ramifications of the pelvis and vessels so that they can be pursued into the substance of the kidney and cut entirely away.

When the total fat in an animal's body is to be estimated, either without or after removing any of the organs, the carcase can be heated without subdivision in a covered basin with the requisite amount of strong potash, 30 to 50 c.c. for 100 grms., till entirely disintegrated, the bones readily rubbed to a fine powder with a pestle, and, after the saponification has been completed by the addition of alcohol, the whole washed into a measuring flask, and an aliquot part taken for treatment, after acidification, with petroleum ether.

CHAPTER III.

DETERMINATION OF THE CHARACTER AND PROPERTIES OF FATS.

WHEN quantities of a specimen of fat are obtainable, which for analytical purposes are unlimited, as is the case in the technical examination of commercial fats, many properties can be, and are, profitably determined which, in physiological investigations, owing to the comparatively small amounts available, often cannot be determined, and at the same time, great as their practical significance may be in technical chemistry, do not convey to the biologist the information which he most requires. Much fuller accounts, therefore, of the determination of such properties as these will be found in technological books than would be in place here.

The methods that may be employed for examining and determining the properties and character of a fat will be dealt with here under the three following heads:—

A. Methods for determining physical properties.

B. General chemical methods in use in technical analysis of fats.

C. Methods for the separation, identification and estimation of the constituents of a fat.

A. PHYSICAL PROPERTIES OF FATS.

1. *Specific Gravity.*

The specific gravity of fats and oils can be determined with different forms of pycnometer or with small amounts by suspending drops in different mixtures of alcohol and water of known graded specific gravities. The pycnometer used by Ubbelohde, with capillary side tube rising from low down in the bottle, can be used for liquid oils and also for solid fats; in the latter case the bottle is filled with water and weighed, and then a weighed amount of the solid is introduced, the stopper inserted, and the diminution of weight plus the weight of the solid fat gives the amount of water displaced and the volume of the fat. It may be noted that the presence of glycerides of lower fatty acids raises the specific gravity of a fat. Butter has at 100° C. the specific gravity 0·868, water at 15° C. being 1; tallow, 0·860; and cocoa-nut oil, 0·873; whereas cacao butter gives the figure 0·857. Rancidity is accompanied by an increase in the specific gravity; in the case of olive oil, in six months' exposure to light and air the specific gravity at 15° was observed to rise from 0·9168 to 0·9246. Remarkably low specific gravity values are characteristic of the liquid waxes, *e.g.*, sperm oil, 0·830 at 100° C.

2. *Melting Point.*

The melting point of fats has been frequently used in physiological experiments for the purpose of characterising a fat, and sometimes to good purpose. It has been noted, for instance, that cows fed on certain kinds of oil yield butter with so low a melting point as to be unmarketable, and the inference drawn that the fat secreted by the mammary gland is the same fat that the animal has had in its food, not fat that has been made by the gland expressly for its secretion, a conclusion for which, of course, there is now other and stronger evidence. Again, Rosenfeld states that geese fed on potatoes yield a fat with a considerably higher melting point than goose fat generally exhibits, and cites this in support of his belief that fat synthesised from carbohydrate food in the animal body is comparatively rich in the glycerides of the saturated acids.

For technical purposes the melting point is determined very frequently, and in some cases this determination is of considerable service. The melted fat is drawn up into a capillary tube, which is then sealed so as to give a column of about 1 cm. in length. The tube should be of about 1 mm. bore, and after it has been filled at least a day should be allowed to pass before it is used for a determination; or a piece of the fat is placed on mercury in a vessel suspended in water that is heated, and the thermometer immersed in the mercury gives the temperature at which the fat spreads out over the mercury.

But since fats are not single substances but mixtures of different glycerides, the melting points are not sharp. And attempts are made to determine the point at which fusion begins and separately the point at which it is complete. For this purpose a capillary is made with a bulbar enlargement on it. In this enlargement with the capillary in a horizontal position a drop of the melted fat is allowed to solidify, and then after one or two days with the capillary vertical in the usual way the temperature is gradually raised. When the drop slides from its lateral position to the bottom of the bulb the point of incipient fusion is taken; when the melted fat runs down into the lower part of the capillary another reading is taken, the point of complete fusion.

In all determinations of the melting point of fats it is necessary to wait some time, not less than one day, before fat which has been melted—for the purpose of filling a capillary, for instance—is used for such a determination. For even pure glycerides of fatty acids that are single chemical entities melt at a much lower temperature if they have been recently melted than that at which they melt if they have been kept in the solid state for some time. The explanation of this phenomenon is not clear, but it has been attributed to the existence of the fat in two forms—an unstable and a stable. The passage from the unstable to the stable form occurs spontaneously but slowly, unless accelerated by the addition of a crystal of the stable glyceride (*cf.* Grün and Schacht, Bömer, Guth).

It is clear from all this that the melting point is not a constant that can be relied upon for characterising a fat in scientific work except under special conditions.

3. *The Solidification Point and Titre Test.*

When a melted fat or oil is cooled down the temperature falls gradually to a variable degree, then rises rapidly to a certain constant temperature, at which it remains steady for a time before it begins to fall again. This steadily maintained temperature is the solidification point.

The determination of this point can be made with a considerable degree of accuracy, and in commercial analysis is very largely used. It is generally carried out on the fatty acids obtained by saponification of the fats, and is then officially known as the Titre test. In different countries somewhat different prescriptions are given for the carrying out of this test, and the results obtained vary according to the method followed. The method described by Dalican is in one form or another in use in Great Britain, the United States and France. The form prescribed by the Association of Official Agricultural Chemists in the United States is briefly as follows: 75 grms. of the fat is saponified in a metal dish with 60 c.c. of 30 per cent. caustic soda solution and 75 c.c. of 95 per cent. alcohol or 120 c.c. of water. It is evaporated to dryness, dissolved in 1 litre of water, and boiled to remove alcohol. The fatty acids are separated by adding 100 c.c. of 30 per cent. sulphuric acid

and heating till clear. They are washed with hot water till free from soluble acids, and then filtered through a dry filter on a hot-water funnel, dried for twenty minutes at 100°, and cooled down to within 15° to 20° C. of the solidification point. They are then poured into a test tube 25 mm. in diameter and 100 mm. long, which is suspended by a cork in the mouth of a jar 70 mm. wide and 150 mm. high. A thermometer graduated in tenths of 1° C. between 10° and 60° is used, the bulb of which is 3 cm. long by 6 mm., and this serves also as a stirrer; the determinations should not vary by more than one-tenth of 1° C.

It is important that the fatty acids should be efficiently dried. Overcooling does not always occur, but in that case the same steadily maintained temperature can be observed, not preceded by a rise of the mercury.

A method that requires such a large amount of material is not likely to be much used in purely physiological investigations.

4. *The Refractive Index.*

The refractive index of fats and oils varies pretty widely, and its determination is made use of in the detection of adulteration of butter, but has not as yet found use in purely scientific physiology.

5. *The Rotatory Power.*

The rotatory power of fats and oils is often due to the presence of cholesterol and other impurities in them. But a triglyceride containing different fatty acids is capable of stereoisomeric modifications, and some of the fatty acids—in Chaulmoogra oil for instance—may themselves be asymmetric substances. Lecithine and glycerophosphoric acid are optically active. But as yet the optical properties of the fats from different animal organs have been very little studied.

B. GENERAL CHEMICAL METHODS IN USE IN THE TECHNICAL ANALYSIS OF FATS.

There are several well-recognised chemical methods of examining and characterising a fat in common use in technical chemical analysis which are also of service in the study of fats in physiology.

1. *The Acid Value.*

The acid value of a fat is obtained by titrating any free fatty acid that it contains and determining the number of milligrammes of caustic potash required to neutralise a gramme of the fat. It is therefore a measure of the degree of hydrolysis of the fat, which may be due to rancidity or to ferment action. A weighed amount of the fat is dissolved in neutral alcohol or alcohol ether, and a few drops of a 1 per cent. solution of phenolphthalein in alcohol added, and the titration effected with a tenth or half-normal aqueous alkali, according to the amount of fat available and the amount of free acids it contains. Under ordinary circumstances animal fats have a very low acid value, and therefore 5 to 10 grms. must be taken for an estimation of this value. The end point is a pink colour that lasts two minutes, though after that it will probably disappear. If the fat is dark coloured phenolphthalein cannot be used, but the titration may be effected by using the "alkali blue 6*b*" of Meister, Lucius and Brüning. A 2 per cent. solution in 90 per cent. alcohol is prepared and dilute potash added till the blue colour just disappears.

2. *The Saponification Value.*

The saponification value of a fat is given by determining the number of milligrammes of caustic potash that are neutralised during saponification of 1 gramme of the fat by the total fatty acids that it contains, whether originally combined with glycerol or other alcohol or free.

For this determination an alcoholic potash solution is required which should be about half-normal. The exact titre of this alkali, as it is liable to change, should be determined shortly before use by titration with a half-normal hydrochloric acid solution.

A weighed amount, usually from 1·5 to 2 grms. of the fat, is heated in the flask in which it is weighed with 25 c.c. of the alcoholic potash on a boiling-water bath for half an hour. In the neck of the flask, which should be of Jena glass or some glass that does not give off alkali, is a cork with a long straight glass tube for a condenser passing through it. Another similar flask similarly fitted, containing the same amount of alcoholic potash and heated side by side with the first, serves as a blank control in case the titre should be altered by carbonic acid or in any other way during the heating. The saponification of the fat having been completed, the alkali in each flask is titrated with half-normal hydrochloric acid and phenolphthalein, and the difference between the amounts of acid required

by the two flasks gives the amount of alkali neutralised by the fatty acids contained in and liberated during saponification from the amount of fat taken; from this the saponification value can be calculated.

In the titration of fatty acids it must always be remembered that soaps are hydrolysed by water and therefore react alkaline. This hydrolysis is prevented if sufficient alcohol is present. A simple and safe rule is to see that there should be about as much alcohol present in the solution as there is water when the titration is completed. According to Kanitz, hydrolysis of soap does not occur in 40 per cent. alcohol, so that to aim at equal parts of alcohol and water is to be on the safe side. If, therefore, during a determination of the saponification value of a fat there has been much evaporation of alcohol, it is necessary to add neutral alcohol before titrating, the amount to be added being determined by a rough estimate of the amount of aqueous hydrochloric acid that will be required for the titration.

The saponification value is clearly a measure of the mean molecular weight of the fatty acids entering into the composition of a fat. Fats such as lard or tallow give figures about 195, whereas butter and oils containing glycerides of lower fatty acids, such as cocoa-nut oil or palm-nut oil, give much higher figures, butter 220 to 233, cocoa-nut oil about 250. Similarly, the low saponification values of liver fat and fish-liver oils (cod-liver oil 171 to 189) indicate the presence either of unsaponifiable substances such as cholesterol or of glycerides of acids of higher molecular weight than stearic. In the oils of most cruciferous seeds the saponification value is less than 180 (colza oil sometimes as low as 170) owing to the presence of large quantities of erucin.

The saponification of waxes, with the exception of spermaceti, is not so readily effected as that of fats, and many suggestions have been made for the facilitation of the process in such cases. The alcoholic potash used should contain as little water as possible, and the heating be prolonged for at least an hour or as much as three hours, and be carried out over a flame on wire gauze and with an efficient condenser. This is said to suffice in the case of beeswax and carnauba wax, but for wool wax other more drastic measures must be taken. Lewkowitsch heats in a sealed tube with twice normal alcoholic potash for three hours at 105° C. Kossel and Obermüller proposed the use of sodium ethylate (5 grms. of sodium dissolved in 100 c.c. of absolute alcohol, freshly prepared). Henriques proposed a method of saponification in the cold: the fat is dissolved in petroleum ether and a solution of soda or potash in absolute alcohol added and the mixture left for twelve hours.

The saponification values of waxes are low because the molecular weights of the alcohols as well as of the fatty acids of which they are composed are high. The figure for beeswax is 90 to 98, for wool wax 102, and for spermaceti about 130.

When in the estimation of fat in animal organs a method is employed such as that described on page 59, the fatty acids obtained

may be dissolved in alcohol and titrated, and the mean molecular weight of the acids insoluble in water determined. If the figure obtained is high, as is often the case, it may be partly or entirely due to the presence of cholesterol or similar unsaponifiable substances. The removal of these by shaking the soap solution with petroleum ether in the way described on page 88, and the determination of their amount by weighing the residue left after distilling off the petroleum from the washed solution, makes it possible to make a correction that brings the molecular weight of the fatty acids down, sometimes from as much as 320 to 280.

The *ester value* is nothing more than the difference between the saponification and acid values, and therefore the number of milligrammes of caustic potash required to neutralise the fatty acids present in a fat in the form of neutral esters.

3. *The Iodine Value.*

The iodine value of a fat gives the amount of halogen reckoned as iodine that the unsaturated acids entering into its composition will take up, expressed in percentage of the weight of the fat. Thus triolein (M.W. 884) will absorb six atoms of iodine ($6 \times 127 = 762$) or 86·2 per cent., and its iodine value is 86·2. The iodine values of fatty acids may be obtained similarly, that of oleic acid being 90·1. The saturated acids and their glycerides of course absorb no iodine, and therefore the iodine value of a fat or of a mixture of fatty acids is an index of the proportion of unsaturated and saturated acids present. The acids with unsaturated linkages in more than one place absorb of course proportionately more iodine, so that the iodine value 86·2 which is given by triolein may also be given by many mixtures of glycerides in which saturated glycerides and at the same time glycerides of acids less saturated than oleic acid are present.

For this determination the most convenient method is that of Wijs, for which the solutions required are a titrated solution of iodine monochloride obtained by mixing solutions of iodine trichloride and iodine in glacial acetic acid, a titrated solution of sodium thiosulphate, a solution of potassium iodide about 10 per cent.

9·4 grms. of iodine trichloride are weighed out into a flask of about 300 c.c. capacity, into which is then poured about 200 c.c. of glacial acetic acid; the flask fitted with a cork through which passes a calcium chloride tube is heated on the water-bath till the whole is dissolved. 7·2 grms. of iodine rubbed to a fine powder in a mortar is then washed with glacial acetic acid into another similar flask and similarly heated. The contents of the flasks are poured into a stoppered litre flask and the undissolved iodine heated again with further quantities of acetic acid till all is dissolved. The solution is then stoppered, allowed to cool down, and made up to a litre with acetic acid and titrated on the following day. For this purpose 10 or 20 c.c. of the solution, measured exactly with a pipette, is treated in a

large Erlenmeyer flask with 5 or 10 c.c. of the potassium iodide solution, diluted with about 200 or 400 c.c. of water, and a sodium thiosulphate solution of known strength run in till the fluid is pale yellow, when some starch solution is added and thiosulphate again run in till the blue colour disappears. From the amount of standardised thiosulphate used the amount of iodine in the measured amount of Wijs is calculated. The strength of the iodine chloride solution is likely to alter a little in the first twenty-four hours, but after that should remain very nearly constant for some weeks. It should be restandardised from time to time in any case, but the intervals will depend on the stability of the particular preparation used. In preparing the solution it is necessary to attend especially to the acetic acid. This should be recrystallised, the mother liquor poured off, the crystals melted and again crystallised. By attending to the preparation of the solvent and preventing subsequent absorption of water a solution is obtained that keeps its titre well.

The thiosulphate solution is prepared by dissolving 48 grms. of the salt in 2 litres of water, and after allowing it to stand for a day standardising it by Volhard's method. For this purpose a solution of 3·8657 grms. of potassium bichromate in 1 litre is prepared. Then 10 c.c. of the potassium iodide solution is treated in a 750 c.c. Erlenmeyer flask with 5 c.c. of strong hydrochloric acid and exactly 20 c.c. of the bichromate solution. These fluids are well mixed, and then diluted with about 300 c.c. of water. The strength of the bichromate solution is such that 20 c.c. acidified with hydrochloric acid liberates exactly 0·2 grm. of iodine from the iodide. When titrated, therefore, with the thiosulphate the number of milligrammes of iodine corresponding to each cubic centimetre of thiosulphate is obtained. The thiosulphate solution should alter very slightly, but should be restandardised from time to time.

The estimation of the iodine value is carried out as follows. Some of the fat or fatty acid mixture is weighed in a stoppered flask of 50 to 150 c.c. capacity. The amount taken should depend upon the probable iodine value and the amount of Wijs' solution used. There should be between two and three times as much iodine in the latter as the fat can absorb. If 25 c.c. of the Wijs' solution be used (= about 640 mgrs. iodine), the amount of iodine absorbed by the fat should be about 250 mgrs. So that if the fat be connective tissue fat, with a low iodine value, about 0·4 grm. should be taken; if the fatty acids obtained from an organ are being investigated about 0·2 to 0·25 grm. should be taken for 25 c.c. of the Wijs' solution. The weighed fat is dissolved in 10 c.c. of carbon tetrachloride, which has been proved not to absorb iodine, and when dissolved the measured amount of Wijs' solution added and the flask stoppered. After standing in the dark for from one to two hours, the contents of the flask are poured into a 750 c.c. Erlenmeyer flask, 10 c.c. of potassium iodide run into the former to dissolve the traces of iodine left in it, and the contents then washed quantitatively into the Erlenmeyer flask with water. The volume of fluid obtained should be about 300 c.c. This is then titrated

with thiosulphate, and the calculation made as in the following example :—

Iodine contained in 25 c.c., Wijs	= 645·7 mgrs.
Thiosulphate used in titration = 34·4 c.c.	
1 c.c. Thiosulphate = 12·2 mgrs. iodine	
Unabsorbed iodine = 34·4 × 12·2	= 419·7 mgrs.
Iodine absorbed . . .	= 226·0 mgrs.
Fat taken = 0·378 grms. „ „ . . .	= 59·7 %=iodine value.

The amount of fatty acids or of fat required for this estimation is about the amount obtained in the method of estimation of fat described in an earlier section, and it is convenient when the two estimations are to be carried out to arrange that this should be so ; then the sample of fat weighed in the first estimation can be used directly for the second, that of the iodine value, without having to be transferred to another vessel.

The estimation of the iodine value of fats is very largely used in commercial analysis, and indeed the classification of fats and oils in use in commerce is based upon this value. The drying oils are those that, owing to their containing glycerides of acids of the linoleic and linolenic series, readily undergo oxidation in the air so as to " dry " to a varnish, and therefore till they have undergone such oxidation can absorb large amounts of iodine, and have high iodine values. According to their iodine values, oils are divided into drying, semi-drying and non-drying oils. Certain animal fats and oils, however, have high iodine values without the property of forming varnishes on exposure to air; salmon oil, for instance, and cod-liver oil have iodine values about 160.

Most of the iodine values given in technological handbooks have been estimated by the older method of von Hübl. In this method the operation lasts much longer, and the iodine solution used is a solution of 25 grms. of iodine in 500 c.c. of 96 per cent. alcohol, mixed the day before being used with an equal quantity of a solution of 30 grms. of mercuric chloride in 500 c.c. of 96 per cent. alcohol. Waller recommended a solution that keeps its titre better than von Hübl's, obtained by adding to 1 litre of the mixed iodine and mercuric chloride solutions 50 c.c. of strong hydrochloric acid. The hydrochloric acid prevents the reaction between iodine monochloride and water ($ICl + H_2O = HCl + IOH$). The values obtained with Wijs' solution are in some cases higher than those obtained with von Hübl's or Waller's, in the case, for instance, of linseed oil, and remarkably so in that of cholesterol.

It will be noticed that the amounts of iodine trichloride and of iodine used in preparing Wijs' solution are not in molecular proportion. Lewkowitsch prepares the solution by dissolving 7·9 grms. of iodine trichloride and 8·7 grms. of iodine in glacial acetic acid, that is to say, molecular proportions, as required by the equation $ICl_3 + I_2 = 3ICl$.

4. *The Hehner Value.*

The Hehner value gives the percentage of fatty acids insoluble in water that are yielded on saponification by a fat.

A weighed amount, from 1 to 4 grms., of the fat is saponified with alcoholic potash, the soap solution washed with hot water into a beaker and made acid with dilute sulphuric acid. The beaker is kept on a hot bath till the subjacent aqueous layer is clear, and then the contents are filtered through a weighed filter. The filter should be carefully selected, but most of the better-class filter papers serve. It should be folded so as to insure rapid filtration, and be half filled with water before the fatty acids are poured on. The beaker is washed with a jet of hot water on to the filter and the fatty acids washed on it similarly so long as any acid reaction can be detected in the washings. The washing should be carried out continuously, so that the filter is kept filled with water. The funnel and filter can then be immersed in a beaker of cold water, so that the fatty acids solidify, and then allowed to drain dry; the filter is then dried and weighed in a weighing bottle, or when dried may be extracted with petroleum ether in a soxhlet apparatus, the petroleum evaporated and the residue dried and weighed.

In many cases this operation presents no difficulties; but the fatty acids obtained from some fats are apt to pass through even the most carefully selected and most carefully handled filter, and then special measures must be adopted. The drops that have passed through may be allowed to solidify and filtered through another filter; the two filters dried and extracted in a soxhlet with some solvent with which the funnel or other vessels to which particles of the fat have adhered have been washed. The solvent can then be evaporated in a weighed flask and the dried residue weighed.

The Hehner value of triolein would be 95·7, that of lard and most oils or fats is about 95, while that of butter is between 86 and 88, and that of cocoa-nut oil is sometimes lower still, in both cases because of the solubility in water of the lower fatty acids which occur in the glycerides present in these fats.

The fats that occur in animal organs yield relatively small Hehner values, because they contain phospholipines.

Lecithine yields only about 70 per cent. of its weight of fatty acids and other phospholipines less still. The estimation, therefore, of the total insoluble fatty acids in the fat extracted from an organ or tissue may serve to give an idea of the nature of its composition. For this purpose about a gramme of the extract weighed in a small flask can be saponified in the flask; if it be desired it can first be titrated, and then saponified with a titrated amount of alcoholic potash so as to obtain its "acid" and "saponification values" at the same time; and then the soap dissolved and washed with water into a flask, such as that described above (p. 59), acidified and shaken with petroleum ether, and finally an aliquot part of the petroleum solution taken as in the quantitative method there described. The extract of the liver of normal animals ordinarily yields from 60

to 70 per cent. of fatty acids insoluble in water, whereas that of a fatty liver may yield more than 90 per cent.

5. *The Reichert Meissl Value.*

The Reichert Meissl value is a measure of the amount of lower fatty acids entering into the composition of a fat which volatilise in a current of steam. The value is expressed by the number of cubic centimetres of 0·1 N alkali required to neutralise the volatile fatty acids liberated under certain prescribed conditions from 5 grms. of the fat or oil. The conditions prescribed in different countries are different. In England the Wollny modification has been adopted by the Government laboratories and the Society of Public Analysts. In this operation 5 grms. of the fat is introduced into a flat-bottomed flask, with a neck 2 cm. in diameter and 7 to 8 cm. in length, and treated with 2 c.c. of a solution of 98 per cent. caustic soda in an equal weight of water, which should be kept protected from carbonic acid, and 10 c.c. of 92 per cent. alcohol. The flask is heated on a boiling bath for fifteen minutes under a reflux condenser, and then the alcohol allowed to evaporate off completely by further heating for at least half an hour without condenser. Then 100 c.c. of water that has been kept boiling for at least ten minutes to remove carbonic acid is added and the flask heated till the soap dissolves. Forty c.c. of normal sulphuric acid and some bits of pumice or porous clay plate are added, and the flask is then at once connected with a condenser tube 8 mm. in diameter, surrounded by a water jacket 35 cm. long, by means of a bent tube 15 cm. long from the cork of the flask to the bend of the tube and 7 mm. in diameter, on the middle of which a bulb 5 cm. in diameter is blown. The flask is heated on an asbestos board 12 cm. in diameter, with an opening in its centre 5 cm. in diameter, with a small flame, till the insoluble acids are melted, and then more strongly. In the course of half an hour 110 c.c. of distillate should be collected and the distillation is stopped. The distillate is well mixed; 100 c.c. measured off, titrated with 0·5 c.c. of 1 per cent. alcoholic solution of phenolphthalein and decinormal soda or baryta solution. A blank test carried out with the same quantities of everything, but without the fat, gives a correction which should not amount to more than 0·2 to 0·3 c.c. The number of cubic centimetres used multiplied by 1·1 gives the Reichert Meissl Wollny number.

Leffmann and Beam's modification of the method is more expeditious, and consists in using 20 c.c. of glycerol instead of the alcohol used by Wollny. After heating with the glycerol and soda for eight minutes the fluid becomes clear and is allowed to cool to 80° C.; 90 c.c. of water, at about the same temperature, and 50 c.c. of 2·5 per cent. sulphuric acid are added. The rest of the process is as in the description given above. This is the official method in Germany.

Clearly such estimations have a comparative value only, and even that only if the same conditions are always observed.

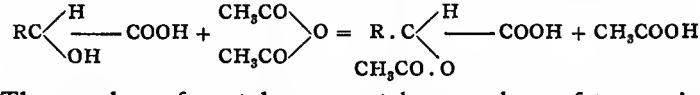
By sending a current of steam through the acid mixture figures that are 25 per cent. higher are obtained, but owing to the decomposition of non-volatile acids by prolonged heating a source of error is introduced if the attempt be made to repeat the distillation by adding more water till the distillate no longer reacts acid.

Most fats and oils contain only traces of volatile acids or their glycerides in the fresh state, and give values therefore that are less than 1. Butter is the most notable exception, with the value 26 to 32. Other exceptions are cocoa-nut oil, Reichert Meissl Wollny value 5 to 8, and dolphin and porpoise oils, the Reichert Meissl Wollny value of which may rise above 60. On the other hand, rancidity is characterised by a rise of this value, as also is the change that accompanies the process of "blowing" oils.

6. *The Acetyl Value.*

The acetyl value gives the number of milligrammes of caustic potash required to neutralise the acetic acid liberated when 1 grm. of acetylated fat or fatty acids is saponified.

If in a fat or oil some of the fatty acids are hydroxy acids, they will when heated with acetic anhydride become acetylated :—



The number of acetyl groups taken up by a fat or mixture of fatty acids will clearly depend on the amount of hydroxy acids present and the number of hydroxyl groups they contain. When by saponification the acetyl groups are split off again as acetic acid, the determination of the amount of acetic acid so liberated by a given quantity of the acetylated product will be a measure of the amount of these hydroxyl groups.

The determination is carried out as follows: 5 to 10 grms. of the fat or mixture of fatty acids is heated with twice its weight of acetic anhydride in a round flask with an inverted condenser for two hours. The contents are then transferred to a beaker of 1 litre capacity, and 500 to 600 c.c. of boiling water added. It is heated for half an hour, while a slow stream of carbonic acid is led through to prevent bumping; salt may be added if the oily matter does not separate readily. Separation is then allowed to take place, and the aqueous layer syphoned off, more water added and syphoned off till the acetic acid formed from the excess of anhydride has been all removed. The oily acetylated product is then filtered through a dry filter in a drying oven to remove water.

A weighed amount, 3 to 5 grms., is then saponified with a known amount of alcoholic potash, and the saponification value determined. The soap is then freed from alcohol by evaporation, and the acetic acid estimated by either of the following methods :—

(1) *Distillation Method.*—An excess of 10 per cent. sulphuric acid is added, and the liquid distilled in a current of steam. The

distillate collected till 100 c.c. requires no more than 0·1 c.c. of decinormal alkali to neutralise it; about 600 to 700 c.c. of distillate will generally be obtained. The total amount of decinormal alkali used to neutralise the distillate, with phenolphthalein as indicator, multiplied by 5·61 and divided by the amount of acetylated product taken, gives the acetyl value.

(2) *Filtration Method.*—The soap solution is treated with the amount of standardised sulphuric acid that exactly corresponds to the alcoholic potash fixed during saponification of the acetylated product, as shown by the determination of the saponification value; the mixture is heated on the bath till complete separation has taken place, and then filtered and washed with hot water till the aqueous filtrate no longer comes through acid; the filtrate then titrated with phenolphthalein gives the acetyl value.

Animal fats do not as a rule contain hydroxy acids that would give an acetyl value. But the presence in a fat of free cholesterol or other alcohols insoluble in water would enable acetylation to occur and cause the fat to have an acetyl value. Certain vegetable oils, however, notably castor oil, contain glycerides of hydroxy acids. Ricinolein, which occurs in castor oil, would itself, if pure, have the acetyl value 159; castor oil commonly shows the value 153 to 156, and grape-stone oil the value 144.

Besides free insoluble alcohols, other substances present in a fat may account for an acetyl value that is not due to hydroxy acids. Partial hydrolysis may have caused the liberation of mono- or di-glycerides which would be acetylated by the acetic anhydride and give products insoluble in water. In a fat, therefore, that has been partially hydrolysed an acetyl value may be obtained which is not due to hydroxy acids. Similarly, a fat that contains glycerides of those lower fatty acids which are soluble in water would, when saponified in the operation of determining the acetyl value, give off the soluble fatty acids to the wash water, and they would not be distinguished from acetic acid liberated from acetylated hydroxy acids. If these lower fatty acids are known to be present, and their amount is known, a correction may be made, and the "true acetyl value" obtained, according to Lewkowitsch, as distinct from the "apparent acetyl value," which is obtained when this correction is not made.

C. SEPARATION, IDENTIFICATION AND ESTIMATION OF THE SEVERAL CONSTITUENTS OF A FAT OR OIL.

The methods that are available for the separation, identification and estimation of the several constituents of a fat or oil depend very largely on those simple properties which underlie the six methods by which the characteristics of a fat or oil are commonly determined in technical analysis, which form the subject of the last section. But there are other methods besides these that must be resorted to when the examination of a fat is to be complete, and indeed at the present time a complete determination of the composition of many kinds of fat is not yet practicable by any of the methods that have been devised.

It is, for instance, in many cases easy to separate neutral fat from free fatty acids by dissolving 5 to 10 grms. of the fat in 50 to 100 c.c. of petroleum ether, adding 25 to 50 c.c. of absolute alcohol and titrating with alcoholic potash and phenolphthalein, then adding as much water as alcohol, so as on shaking to get a separation of a solution of soap in 50 per cent. alcohol from a solution of neutral fat in petroleum. If these solutions are washed by shaking, the soap solution with fresh petroleum, and the neutral fat solution with fresh 50 per cent. alcohol, and adding the washings to the corresponding solutions, the whole of the fatty acids originally present in the fat in the free state or in the form of soap can be obtained from the alcoholic solution by acidifying and shaking out with petroleum ether.

Similarly, in the petroleum solution, which contains the neutral fats and any cholesterol or other unsaponifiable substances that may be present, these latter are obtained by saponifying the fat with alcoholic potash, dissolving the product in equal parts of alcohol and water, and shaking the solution with petroleum ether. The saponified fats will be contained in the alcoholic solution and the unsaponifiable matter in the petroleum ether. If, however, the original fat contains phospholipines a solution of it in petroleum is likely to give up to 50 per cent. alcohol some of these phospholipines as well as any soap that is present in the mixture.

The subject-matter, therefore, of this section resolves itself primarily into three divisions—the separation, identification and estimation of—

1. The various fatty acids (pp. 75-85); and
2. The various alcohols, that enter into the composition of the fat;
3. The phospholipines, so far as the present state of our knowledge of these substances permits (pp. 90-105).

1. *Separation of the Fatty Acids.*

The fatty acids that enter into the composition of a fat are obtained by saponifying the fat with alcoholic potash. For 100 grms. of the fat it is convenient to take the equivalent of about 40 grms. of potash (80 c.c. of a solution obtained by dissolving 500 grms. of potash sticks in 500 c.c. of water) and 100 c.c. of alcohol. The mixture is heated for half an hour at or near its boiling point on the water-bath, and then after most of the alcohol has evaporated dissolved in a large beaker in hot water, of which about 2 litres should be taken. The soap solution heated on the bath will lose the remainder of its alcohol if that is desired, and then 100 c.c. of 40 per cent. sulphuric acid is stirred in and the heating continued till the fatty acids separate to the top, leaving the aqueous acid solution clear below. This is then syphoned off, or if on cooling the fatty acids solidify, can be poured off. The fatty acids are then heated again with fresh changes of water till the water shows no reaction with congo paper and the mineral acid has been removed.

When there is reason to suppose that volatile or soluble fatty acids are present in the fat, when, *i.e.*, the saponification value is high, the sulphuric acid should be added in a flask so that the volatile acids can be distilled off in a current of steam.

The non-volatile acids left after the distillation of the volatile acids can then be washed repeatedly with water to remove sulphuric acid and dried for further examination. When there is reason to suppose that the insoluble acids so obtained contain unstable unsaturated acids, when, *i.e.*, the iodine value is high, the fatty acids should be dissolved in petroleum, the solution dried with anhydrous sodium sulphate, filtered, and the fatty acids kept in petroleum solution in stoppered bottles till further steps are taken for examining them.

The fatty acids so obtained will necessarily contain as mentioned before cholesterol and any other unsaponifiable matter which is soluble in petroleum, and before attempting a further separation and characterisation of the acids such substances should be removed by converting the acids into soaps and extracting either the dried soaps or a solution of them with some solvent such as ether or petroleum ether according to the methods described later.

The *mean molecular weight of the acids* may be taken when this has been done. As much of the petroleum solution as will contain about 0·5 grm. of fatty acids is measured off into a weighed round flask with a short, wide neck and of about 150 c.c. capacity. The flask is fitted to a condenser with a bent tube, and has leading through its cork another tube connected with an apparatus for delivering carbonic acid. It is filled with carbonic acid and the petroleum distilled off on a water-bath. When the distillation is apparently finished the receiver is connected with a pump and exhausted, and the heating continued in a good vacuum for half an hour. The flask is then removed, allowed to cool in a desiccator

and weighed. The fatty acids are dissolved in the flask in about 20 to 25 c.c. of alcohol, and after adding a few drops of 1 per cent. alcoholic solution of phenolphthalein are titrated with 0·1 N alkali. The amount of alcohol taken for dissolving the acids should be not less than the amount of aqueous alkali used for the titration, so that the final concentration of the alcohol should be sufficient to prevent hydrolysis of the soaps, *i.e.*, about 50 per cent. The weight of fatty acids taken in milligrammes divided by the number of cubic centimetres of decinormal alkali used and multiplied by ten gives the molecular weight (*e.g.*, 0·284 grm. stearic acid would require 10 c.c. decinormal alkali to neutralise it, and the molecular weight of stearic acid is 284). In some cases there may be difficulty in determining the end point with phenolphthalein if the colour of the acids is dark, and then a 2 per cent. solution of the "alkali blue *6b*" of Meister, Lucius and Brüning may be used, or the same end may be attained by diluting the solution with more alcohol. If high values for the molecular weight are obtained this should lead to the suspicion that cholesterol and unsaponifiable substances are present in considerable quantities, and after complete removal of these lower values will in that case be observed.

In this way the acids will have been separated into two portions, one volatilised by a current of steam contained in the aqueous distillate, the other non-volatile and insoluble in water.

Solubility of Volatile Fatty Acids in Water.

	100 c.c. dissolve at 15° C.	At 100° C.
Acetic and Butyric .	∞ grms.	— grms.
Valeric . . .	3·5 ,,	— ,,
Caproic . . .	0·882 ,,	— ,,
Caprylic . . .	0·079 ,,	0·250 ,,
Capric . . .	— ,,	0·1 ,,

The volatile acids may consist of the acids soluble in water—formic, acetic, propionic and butyric—and of acids of higher molecular weight, more or less insoluble in water, up to and including traces at any rate of lauric and myristic acid, if they are present. When caproic or higher volatile acids are present they will be seen floating on the aqueous distillate as oily drops, or even as solid flakes. As a general principle it is the first portions of the distillate that contain the less soluble volatile acids of higher molecular weight.

Duclaux distilled ten fractions of 10 c.c. each from 110 c.c. of saturated solutions of butyric, caproic and caprylic acid respectively, and found that, while 75 per cent. of the caprylic was contained in the first two fractions, three fractions had to be collected before this amount of the caproic acid was recovered, and six fractions before the same amount of the butyric acid could be obtained. The total yield of caproic and caprylic acids in the ten fractions was 100 per cent., of butyric 97·5 per cent.

But only a very imperfect separation of the volatile acids can be effected by collecting the distillate in fractions. The total amount

of acid in the distillate can be titrated and the solution obtained evaporated down. To attempt to determine the mean molecular weight of the acids by evaporating down a neutral solution to dryness and weighing the residue would lead to no certain result owing to hydrolysis of the salts in dilute solution and loss by evaporation of some of the acids, so that it is best to add excess of alkali before concentrating the distillate and to attempt to identify and separate the acids subsequently. The alkaline salts when dry may be treated with a small excess of 20 per cent. sulphuric acid. Caproic and higher acids will then separate as an oil. The subjacent aqueous solution may be treated with salt, and butyric acid will then separate out from the salt solution also as an oil. And the salt solution shaken with ether will give up its lower acids to the ether. Silver calcium or barium salts of the several fractions of acids so obtained may be made, and from the amount of metal these salts contain the composition of the acids in each fraction determined. The amides of the several acids may also be prepared and identified by their melting points.

When volatile acids higher than caproic are present it may be worth while to collect the first portion of the distillate separately, dissolve the insoluble acids that it contains in ammonia and precipitate the silver salts with silver nitrate, collect the precipitate, dry it and estimate the silver in it. The later fractions of the distillate containing only soluble acids may be made to yield silver salts by heating the solution with silver carbonate, filtering hot and crystallising out in the cold.

When the amount of volatile acids obtained as sodium salts is sufficient to allow it, the best method of separating them is of course to shake out the acidified mixture with ether, evaporate off the ether and fractionate the residue by distillation. Each fraction is weighed, its boiling point noted, and salts or amides prepared and analysed.

In his study of the products of fermentation Nencki made use of the formation of guanamines, on heating guanidine salts of volatile fatty acids to 220° to 230° C., for the identification of these acids. The guanamines crystallise readily and can be identified by their crystalline form.

The separation of the non-volatile acids into the two groups of *the solid saturated acids and the liquid unsaturated acids* is generally effected by the conversion of the acids into their lead salts and the extraction of these with ether. Broadly speaking, the lead salts of unsaturated acids are soluble, those of saturated acids insoluble, in ether. The method may be used for the purpose of quantitatively determining the approximate proportions in which these two groups of acids occur in a fat, but is also of value for the separation of the groups of acids with a view to the further study of the individual components of each of the two groups.

The method was first proposed by Gusseroff in 1828, but is generally known as Varrentrapp's method, as he was the first some years later to use it for the preparation of oleic acid. For quantitative purposes it has undergone many slight modifications since that time.

From 2 to 4 grms. of fat is saponified in a 300 c.c. flask with 50 c.c. of about half-normal alcoholic potash, and the solution having then been acidified with acetic acid in the presence of phenolphthalein, alcoholic potash is run in till the neutral point is reached, and water added so as to make about 100 c.c. in all. The lead salts are precipitated by adding gradually and with continual shaking a boiling mixture of 30 c.c. of 10 per cent. lead acetate solution with 150 c.c. of water. The lead soaps will adhere to the sides of the flask, and after cooling down the water can be poured off clear, if necessary through a filter. Any loose particles on the filter are returned to the flask, which is then again filled with boiling water and allowed to cool; the water is then poured off and the washing repeated. Finally, adherent drops of water are removed with filter paper, and without any further drying of the soaps 150 c.c. of ether is poured on, and with continual shaking the ether is warmed under a condenser till they are loosened from the flask and the insoluble part settles as a powder beneath the ethereal solution. After cooling down the solution is poured through a filter into a separating funnel, and the undissolved soaps washed with ether on to the filter; the filtrate and washings are then shaken with dilute hydrochloric acid to decompose the lead salts. When separation has taken place the lead chloride and acid water are drawn off and the ethereal solution of fatty acids washed with small quantities of water till the washings are neutral. Finally the ether is evaporated from a weighed round-bottom flask filled with carbonic acid gas and finally exhausted in a hot water-bath.

From the insoluble lead soaps the solid saturated fatty acids can be similarly recovered. If the iodine value of these acids be taken an idea of the success of the separation can be formed. Theoretically it should be zero; practically it may be as low as 3 or 4, but often is higher.

The separation as a matter of fact is not exact. The lead salts of even the higher saturated acids are not entirely insoluble in ether, and in the presence of unsaturated acids salts are formed in which the dibasic lead combines with an atom of saturated and an atom of unsaturated acid. Moreover, the lower saturated acids give lead salts which are soluble in ether, so that when these acids are present, as, for instance, in cocoa-nut oil, the iodine value of the acids obtained from the soluble lead salts is low—as low as 36 it may be. It is also to be remembered that some unsaturated acids, erucic and isoleic, form salts with lead which are very slightly soluble in ether.

Another method proposed by Farnsteiner, and recommended by some on account of its convenience, effects a separation of the same sort as Varrentrapp's method with a similar degree of accuracy. The lead soaps prepared in the same way are dissolved in warm benzene, of which 50 c.c. are required for the acids obtained from 1 grm. of oil. In this solvent they all dissolve, but the salts of the saturated acids separate out on cooling to 10° C. in crystalline form. The supernatant benzene solution is drawn off by means of a small inverted thistle funnel with close-meshed silk tied over the mouth, the

other end being bent so as to pass through the cork of a filtering flask connected with a water-pump; the crystals are washed a few times with 10 c.c. of benzene cooled to 10° C., and then redissolved in 25 c.c. of warm benzene and recrystallised. The whole process is repeated, and the crystalline lead salts finally dried and weighed; the benzene solution is evaporated, and the soluble lead salts also dried and weighed. The results obtained by Farnsteiner showed that it was possible to get figures correct within 2 or 3 per cent. of the theoretical.

The principles of the lead salt ether or lead salt benzene method can be used for the separation of solid and liquid fatty acids on a larger scale when the examination of the components of either of these groups of acids is contemplated. But as a rule the ether method, requiring a smaller volume of solvent, will be preferable. In any case the iodine value of the fractions so obtained should be taken and compared with that of the original mixture of acids so as to see how far the separation has been successful.

The saturated acids obtained from the lead salts insoluble in ether or benzene can be resolved into their constituents by use of the following methods:—

(1) Fractional precipitation of magnesium or barium salts (Heintz). The acids are dissolved in sufficient alcohol to prevent separation on cooling, and while hot a solution of magnesium acetate in alcohol is added which contains only one-fifth or one-tenth of the amount calculated to be necessary for complete precipitation. The precipitate is separated and the filtrate treated with sufficient ammonia to neutralise the liberated acetic acid, and then again with the same amount of magnesium acetate. In this way a series of fractions is obtained from which the free acids are separated by means of petroleum ether and dilute hydrochloric acid. The melting point and molecular weight of the acids from each fraction is taken, and it will be found if more than one solid acid was present that the acids of higher molecular weight and melting point occur in the earlier fractions. By this method, at any rate when only two solid acids are present, a separation and identification of the acids is possible, especially if the acids are crystallised from alcohol a few times.

For acids of lower molecular weight barium acetate is used in preference to magnesium acetate.

(2) Fractional distillation of the acids or their methyl or ethyl esters *in vacuo* has frequently proved useful as a means of separating and isolating pure substances. The boiling points that have been observed with the corresponding pressure readings will be found in the tables of the saturated and the unsaturated acids on pages 10 and 11 (Krafft and Ubbelohde).

In addition to the above methods, which are necessarily long and laborious, and require large quantities of material, certain methods have been devised for detecting or estimating individual acids that should be mentioned.

Arachidic acid, of which pea-nut oil contains about 5 per cent., can be detected in olive oil that has been adulterated with not less than 10 per cent. of the former oil, and contains therefore 0·5 per cent. of this acid, if the fatty acids from 10 grms. of the oil are dissolved in 50 c.c. of hot 90 per cent. alcohol (specific gravity, 0·818 at 20° C.), and cooled down to 15° C. The crystals obtained are washed with measured quantities of 90 per cent. alcohol, and then with 70 per cent. alcohol, and finally dried and weighed; a correction being made for the amount of 90 per cent. alcohol used on the basis of the solubility of arachidic acid or rather of the mixture of this acid with lignoceric acid that occurs in pea-nut oil (100 c.c. 90 per cent. alcohol dissolves 22 mgrs. at 15°, 45 mgrs. at 20° C.). The acid may be identified by its molecular weight.

Stearic acid is estimated by the method of Hehner and Mitchell, which consists in crystallisation from a previously prepared solution of pure stearic acid in 95 per cent. alcohol saturated at 0° C. For this 3 grms. of stearic acid are heated with a litre of the alcohol in a stoppered bottle, which is then placed in ice water at 0° C. for twelve hours. Without removing the bottle the saturated solution is drawn off with the help of a pump through a thistle funnel covered with fine silk or linen. About a gramme of the solid acids to be examined is weighed out exactly, and dissolved in 100 c.c. of the stearic acid solution, and kept similarly in ice water for twelve hours. The flask is then agitated and left for an hour or so longer in the ice water. The solution is then sucked off, as above, with the pump; the flask washed with small amounts of the stearic acid solution at 0° C., and the washings removed as completely as possible. Finally the crystals adhering to the funnel or its covering are dissolved into the flask with a stream of hot alcohol, and after evaporating off the alcohol are weighed in it. A small correction should be made for the stearic acid dissolved in the film of solution unavoidably left after washing the flask and crystals.

In this way the amount of stearic acid contained in certain fats and oils, as given below, was ascertained:—

Butter contains	0·5 per cent.	stearic acid.
Mutton tallow contains	16 – 22 p.c.	,,
Beef tallow ,,	51 p.c.	,,
Lard ,,	6 – 16 p.c.	,,
Cacao butter ,,	39 – 40 p.c.	,,
Cocoa-nut oil ,,	1 p.c.	,,
Palm oil ,,	0·5 – 0·7 p.c.	,,
Olive oil ,,	0 p.c.	,,
Almond oil ,,	0 p.c.	,,

Palmitic acid can be estimated from the mean molecular weight of a mixture of this acid with stearic acid, if it is known that no other acid is present. And it can be isolated from such a mixture, if the mixture is dissolved in enough alcohol to hold in solution at 0° C. the palmitic acid known to be present (100 c.c. 95 per cent. alcohol at 0° C. dissolves 0·56 grm. palmitic acid and 0·12 grm. stearic acid). On cooling the solution to 0° C. for some hours the crystals formed will be principally stearic acid and by recrystallisa-

tion can be purified. The filtrate concentrated to half its volume and treated with enough magnesium acetate solution to precipitate the stearic acid that it contains, will give a filtrate from which the greater part of the stearic acid has been removed, and if a second precipitation with half the first amount of magnesium acetate be carried out the filtrate will yield after recrystallisation from alcohol pure palmitic acid.

For the detection and isolation of lauric and myristic acids, a low molecular weight after removal of volatile acids on the one hand, and fractional precipitation of barium salts on the other, are the only methods other than the more satisfactory fractional distillation.

The unsaturated acids obtained from the lead soaps soluble in ether or benzene may be examined by the following methods :—

(1) *Bromination.*—On treating a cooled solution of the unsaturated fatty acids in glacial acetic acid or chloroform with bromine they undergo bromination and the unsaturated carbon atoms become saturated. The dibromostearic acid formed from oleic acid is soluble in the ordinary solvents for fat, including petroleum ether; the tetrabromostearic acid from linoleic acid is only slightly soluble in petroleum ether but dissolves readily in diethyl ether, while the hexabromostearic acid from linolenic acid is almost insoluble in ether but dissolves in hot benzene. A partial separation at any rate of the bromination products can be effected by means of these solvents, and the composition of a mixture of unsaturated acids approximately ascertained in this way. Thus if the mixed acids have the iodine value 120 and yield no bromination product that is insoluble in ether, *i.e.*, no product with more than four atoms of bromine, it would be safe to conclude in most cases that the mixture was composed of two-thirds oleic acid (iodine value 90 to 91) and one-third linoleic acid (iodine value 181 to 184). For unsaturated acids containing more or less than eighteen carbon atoms have been shown to exist in very few fats or oils.

The *hexabromide test*, which is used in commercial analysis, is an application of this principle of separation. For this test 0·3 grm. of the liquid fatty acids or 1 to 2 grms. of the unsaponified oil is dissolved in glacial acetic acid by itself or mixed with ether, and cooled down to 5° C. Bromine is added drop by drop till the coloration persists. The mixture is allowed to stand at 5° C. for three hours and then filtered. The precipitate is washed with 10 c.c. of ether at the same temperature several times and what is undissolved is dried and weighed.

This residue, insoluble in ether, may consist in some cases of a mixture of hexabromo and octobromo derivatives. Hexabromostearic acid melts at about 180° C., whereas the octobromide from marine animal oils decomposes at about 200° C. without giving a true melting point. Estimation of the amount of bromine it contains will show this, or else the precipitate may be subjected to long extraction with hot benzene. If it does not dissolve in this solvent entirely the presence of an octobromo derivative is indicated, and its amount may be approximately estimated. The fatty acids ob-

tained from linseed oil yield from 30 to 40 per cent. of their weight of "hexabromide," corresponding to from 11 to 15 per cent. of linolenic acid. Octobromides have been obtained from cod-liver oil, herring oil and other marine animal oils, and from the fatty acids of the pigs' liver. The bromination products insoluble in ether obtained by Hartley seemed to consist entirely of an octobromo-arachidic acid; hexabromostearic acid could not be detected.

The bromination products soluble in ether may similarly, by the use of petroleum ether, be resolved into their constituents since tetrabromostearic acid dissolves in this solvent only when heated and crystallises out in needles, M.P. 114-115, almost quantitatively on cooling; 100 c.c. of petroleum ether at 12° C. dissolves from 14 to 21 mgrs. of the tetrabromo derivative of linoleic acid. Dibromostearic acid is a liquid substance easily soluble in all fat solvents, including petroleum ether. It may be identified by its molecular weight, 442, as may also tetrabromostearic acid, M.W. 600, and hexabromostearic acid, M.W. 758. In the case of this last determination the substance is dissolved in 50 parts of benzene at about 75° C., an equal volume of hot absolute alcohol is added, and the titration carried out in the hot liquid (Farnsteiner).

The recovery of the original unsaturated acid from a bromination product was carried out by Hazura in the case of tetrabromostearic acid: 17 grms. of the tetrabromide was boiled for thirty-six hours with 600 c.c. of alcohol and 150 c.c. of strong hydrochloric acid and some tin-foil. The mixture was then diluted with water and shaken with ether, which took up the ethyl ester of linoleic acid.

Tsujimoto obtained similarly by reduction with zinc and alcoholic hydrochloric acid, clupanodonic acid from the octobromide prepared from Japanese sardine oil.

(2) *Identification of unsaturated acids by oxidation and isolation of the acids so formed.* When oleic acid is oxidised at a temperature little above zero with alkaline permanganate, it takes up two hydroxyl groups and is converted into the saturated dihydroxystearic acid. Similarly, under these conditions linoleic acid takes up four hydroxyl groups and linolenic acid six; sativic or tetrahydroxystearic, and linusic or hexahydroxystearic acid, are formed respectively.

The isolation of these oxidation products reveals therefore the presence of the corresponding unsaturated acids. The procedure first devised by Hazura is as follows: 30 grms. of the liquid fatty acids are dissolved in 2 litres of water with the requisite amount of potash, and the solution cooled down to near zero. Into the vessel containing the soaps and standing in a freezing mixture 2 litres of 1·5 per cent. potassium permanganate also cooled to 0° C. is run slowly in a fine stream, while the mixture is mechanically stirred. After standing for a short time the manganese peroxide can be filtered off and a clear filtrate obtained, which is then acidified with sulphuric acid. (Hazura dissolved the peroxide and acidified at the same time by means of sulphurous acid, which is not so convenient if a complete examination of the oxidation products is contemplated.) When the reaction becomes acid unaltered fatty acids and some of

the oxidation products are precipitated. These are filtered off, pressed out and dried on porous plates. They are then extracted with petroleum ether, which dissolves out and removes unaltered fatty acids but not the hydroxy derivatives. The insoluble part is then treated with large quantities of ether (2 litres for 20 grms.). In this the dihydroxystearic acid dissolves, and from the solution it may be recovered and recrystallised from alcohol till its melting point is no longer changed by the process. The tetra- and hexahydroxy acids are insoluble in ether, and the former slightly soluble in boiling water (1 grm. in 2 litres), the latter comparatively easily soluble in cold water. By boiling out therefore the products which do not dissolve in ether repeatedly with large volumes of water and filtering in a hot funnel a solution is obtained from which sativic acid if present will crystallise out as it cools. The large volumes of mother liquor are concentrated down after being neutralised, and when sufficiently concentrated to give a precipitate on acidification made acid; the precipitate collected and dried is extracted with ether to remove lower oxidation products and then crystallised from alcohol. Linusic and isolinusic acid so obtained may be separated by crystallisation from small volumes of water in which the latter is more soluble than the former.

The separation of tetraoxystearic from dioxy and hexaoxy acids can be effected in this way more easily than the isolation of individual acids. The melting points given for the acids from linseed oil when isolated are:—

Dihydroxystearic acid	. . . . .	136·5
Sativic	,, ...	173
Linusic	,, ...	203-205
Isolinusic	,, ...	173-175

But it is not always easy to obtain products that melt true to these data, and even in Hazura's experiments there are indications that the sativic acid fraction contained two acids of different melting points (*cf.* too Hartley). In some cases the dioxystearic acid appears, if obtained from other sources than those drawn on by Hazura, to be a different acid with different crystalline form, different solubilities and different melting point. The acid obtained from isooleic acid melts, for instance, according to Saytzeff at 77° to 78° C.

Where therefore it is desired to identify the particular oleic or linoleic acid that is present in a fat, oxidation with permanganate at low temperatures will probably fail. Information may, however, be obtainable if the further oxidation of the products obtained in cold oxidation be carried out at higher temperatures.

Oleic acid, which yields a dihydroxystearic melting at 136·5 when oxidised at 0° C., yields on heating with permanganate on the water-bath, as does also the isolated dihydroxystearic acid itself, pelargonic and azelaic acid. As mentioned above (p. 17), the group $-C^{H}_{OH}-C^{H}_{OH}-$ in the centre of the chain in this dihydroxystearic acid is the seat of cleavage resulting from its further oxidation and the formation of two carboxyl groups. In this way two

new acids are formed—monobasic, nonylic or pelargonic acid on the one hand, and dibasic azelaic on the other.

To obtain this result oleic acid or the dihydroxystearic acid formed from it is dissolved in the requisite amount of potash, and heated with permanganate solution on the water-bath.

(3) *Fractional distillation of the methyl or ethyl esters* of the fatty acids effects a partial separation of these acids.

By this means Bull isolated from cod-liver oil palmitoleic acid $C_{16}H_{30}O_2$, gadoleic acid $C_{20}H_{38}O_2$, and erucic acid $C_{22}H_{42}O_2$, three unsaturated acids of the oleic series not previously found in animal fats. The methyl esters from 2 kgrms. of cod-liver oil were distilled in fractions at 10 mm. pressure at temperatures from 160° to 240°, the acids prepared from the several fractions were separated, and their properties and those of some of their salts determined (Bull).

The methyl esters of the unsaturated fatty acids from the livers of pigs were prepared by Hartley, and fractionated by distillation at 3 mm. Hg pressure. In this case the results seemed to indicate that the boiling point rose with an increase in the number of doubly bound carbon atoms. Several fractions were collected boiling between the temperatures 150° and 190° C. The lowest boiling fraction had the iodine value 127·5, and as the boiling point went up the iodine value rose correspondingly, and the fraction boiling at 190° showed the value 249. But the true explanation of this phenomenon probably is to be found in the fact that the most highly unsaturated acid present in this case, the acid from which the octobromide referred to above was obtained (p. 82), was an acid of higher molecular weight than the others with a lower iodine value, and for this reason, and not because it was less saturated, boiled higher. For Lewkowitsch in distilling the methyl esters of the acids from cotton-seed oil could not detect any such relationship between boiling point and iodine value, the acids with differing iodine values having in this case probably the same, or nearly the same, molecular weight.

Fractional distillation separates unsaturated acids of different molecular weights, but not acids of similar molecular weights but different iodine values. This fact is illustrated, too, by the fractionation effected by Bull. The boiling point of stearic acid in absolute vacuum is 155°, that of oleic acid under these conditions is given as 153°.

(4) Farnsteiner proposed a method by which he maintains it is possible to separate not only saturated from unsaturated but oleic from less saturated acids. The acids are converted into the barium salts and dried, and then heated with benzene to which 5 per cent. of 95 per cent. alcohol has been added. The unsaturated soaps dissolve, while the saturated do not. On cooling to 11° C. for one and a half hours, barium oleate separates out in crystals almost quantitatively, while the salts of linoleic and linolenic acids remain in solution. This method has not, however, given satisfactory results

in the hands of others. Farnsteiner himself gives among his results the following :—

	Saturated acids, per cent.	Oleic acid, per cent.	Other unsaturated acids, per cent.
Olive oil . .	10·0	70·9	14·9
Pig fat . .	42·2	39·2	13·9
Cacao butter .	59·7	31·2	6·3

The presence of oxy acids is revealed by the acetyl value. They occur in rancid fats and oils, and in such as have undergone oxidation from exposure to the air. In nature castor oil is composed mainly of the glyceride of ricinoleic acid ; wool fat contains lanopalmic and lanoceric acids.

Attempts may be made to separate oxy acids from other fatty acids by means of petroleum ether ; but though an isolated pure oxy acid such as ricinoleic acid may be insoluble in petroleum ether, when mixed with other acids it is liable to remain in solution in petroleum, so that the acids obtained from the filtered petroleum solution may have an acetyl value.

Anhydrides or lactones of fatty acids or oxy fatty acids are detected by comparing the amount of potash required for neutralising the acids with the amount neutralised after boiling them with alcoholic potash, by comparing, that is, the acid and saponification values of the fatty acids. Anhydrides will, of course, react neutral on titration unless they have been reconverted into the acids by boiling with alcoholic potash.

2. *Identification, Isolation and Estimation of Alcohols in Fats.*

(*a*) *The Estimation of Glycerol.*

When a fat is saponified, the soap taken up in water and acidified, the glycerol remains in the acid aqueous fluid; and if this is neutralised and evaporated down to a small volume the presence of glycerol can be qualitatively recognised by the acrolein reaction. On heating some of the concentrated solution with acid potassium sulphate the pungent fumes of acrylic aldehyde, acrolein, are given off, the glycerol having lost two molecules of water.

(1) *The Acetin Method.*

The estimation of glycerol is not infrequently carried out in technical analysis, and is sometimes necessary in biological investigations. Many methods have been proposed, the most generally approved of which is the acetin method. In this procedure the glycerol is converted into triacetin, and the amount of acetic acid so bound determined by its saponification value. If the problem is to determine the amount of glycerol in a fat or oil it is customary to take as much as 20 grms. for the purpose, corresponding to about 2 grms. of glycerol. The fat is saponified in the usual way with alcoholic potash; the soap solution acidified with sulphuric acid and filtered. The filtrate is treated with barium carbonate in excess and evaporated down to a small volume. The residue is treated repeatedly with a mixture of one part of ether and three parts of alcohol, and the filtered alcohol ether evaporated at a low temperature. The crude glycerol, in a round-bottom 100 c.c. flask, is dried in a desiccator, and its weight approximately determined. It is then heated for one and a half hours under a condenser with about 10 c.c. of acetic anhydride and about 4 grms. of sodium acetate that has been well dried in an oven. After cooling down somewhat about 50 c.c. of water is poured down the condenser to wash into the flask traces of adherent triacetin that has volatilised and condensed on the sides of the condenser tube. The triacetin is dissolved in this water with slight warming if necessary, but without detaching the condenser. The solution is filtered into a 600 c.c. flask when cold, and neutralised to phenolphthalein with half-normal aqueous soda which is free from carbonate. The flask must be shaken well while the soda is added and an excess carefully avoided to prevent saponification of the triacetin, the first change in colour being taken as the end reaction rather than the development of a definite pink. 25 c.c. of a 10 per cent. soda solution is then added, and the same amount of the same soda measured with the same pipette introduced into another control flask. Both flasks are heated for a quarter of an hour with condensers on a water-bath and titrated with standard hydrochloric acid (normal or half-normal). The difference between the two titrations gives the amount of acetic acid set free during saponification: 1 c.c. normal acid = $\frac{1}{3}$ mgr. mol. = 30·67 mgr. glycerol (Benedikt, Cantor and Lewkowitsch).

(2) *Oxidation Methods.*

The Benedikt Zsigmondi method, as modified by Herbig- consists in the conversion of glycerol by strongly alkaline permanganate at the ordinary temperature into a molecule of oxalic and a molecule of carbonic acid. The oxalic acid is separated as lime salt and titrated. From 2 to 3 grms. of fat is saponified with potash and pure methyl alcohol, the alcohol evaporated off and the soap taken up in water, treated with hydrochloric acid and warmed. If the fatty acids remain liquid on cooling paraffin may be added to facilitate filtration. The filtered aqueous solution and wash water is neutralised with potash and 10 grms. of potash added in excess, and then a solution of about 5 per cent. permanganate run in till the fluid is no longer green but blue or almost black. The mixture is allowed to stand for half an hour, and enough hydrogen peroxide then added to decolorise the fluid, and no more. It is poured into a litre flask and made up to 1 litre, and 500 c.c. are filtered off. This filtrate is acidified with acetic acid and the oxalic acid precipitated with calcium chloride ; the washed oxalate of lime dissolved in excess of warm dilute sulphuric is then titrated at about 60° C. with decinormal permanganate : 1 c.c. = 4·5 mgr. oxalic acid, or in the total amount used at the outset, 9·2 mgr. glycerol. If the fat, however, contained unsaturated acids which might have undergone oxidation, this method cannot be used, as the products of such oxidation may yield oxalic acid when treated with permanganate.

Oxidation with bichromate in acid solution and titration of the excess of bichromate (Legler and Hehner) has been proposed but it is more liable to error than the oxidation method given above.

(3) *Other Methods.*

A method similar to Zeisel's method for estimating methoxyl or ethoxyl groups, in which by treatment with hydriodic acid glycerol is converted into isopropyliodide and distilled into silver nitrate and the silver iodide weighed (Zeisel and Fanto).

It is possible also to estimate glycerol directly, as done by Shukoff and Schestakoff. The glycerol solution is made slightly alkaline with potash and concentrated at a temperature not above 80° C. till syrupy. The amount taken should be calculated to yield about 1 grm. of glycerol and the evaporation should be carried only so far as to have about the same amount of water present as glycerol. Then 20 grms. of anhydrous sodium sulphate are added, and the mass extracted with acetone in a soxhlet apparatus for six hours. The acetone must be pure and dry and all connections must be of glass. The acetone solution, washed with petroleum ether if oily drops float on it, is evaporated and the glycerol dried at 75° to 80° C. for four hours and weighed in the flask fitted with a stopper. The product is generally coloured.

Fats that have undergone partial hydrolysis may contain *mono- or diglycerides.* These should be revealed by the following procedure : The fat is freed from free fatty acids in the usual way, and its acetyl value determined. The fat must of course contain no glycerides either of hydroxy acids or of soluble fatty acids, or if it does, the amount of these must be determined separately and a correction made.

(*b*) *Isolation and Estimation of other Alcohols.*

The principle on which the separation of cholesterol from fats is carried out was indicated above (pp. 60 and 74).

The fat is saponified and the soaps dissolved in 50 per cent. alcohol are shaken with petroleum ether. The petroleum solution is separated and washed with 50 per cent. alcohol, and the alcohol solution is washed with petroleum ether, each two or three times. The combined petroleum solutions are evaporated and the residue after carrying it through the whole process a second time with smaller volumes of the solvents is finally weighed (Hönig and Spitz).

Cholesterol is less soluble in petroleum ether than in ordinary ethyl ether, so that there are advantages in using the latter solvent for the extraction,[1] and in this case it may be found more convenient to extract the soap solution in a continuous extraction apparatus. One of the simplest and most convenient forms of apparatus for this purpose consists of a cylinder about two-thirds filled with the soap solution, through the cork of which two tubes pass, one reaching down to near the bottom of the cylinder, the other only just through the cork: the former of these tubes is continued straight up and just projects through the upper end of a cork in the mouth of a vertical condenser: the other tube is bent at an angle of about 60° after leaving the cylinder and is then carried by another bend vertically through the cork of a flask on a water-bath in which ether is boiled. The ether vapour passes up from this flask through another bent tube leading from the flask into the condenser from which it is returned to the bottom of the cylinder by the long straight tube.

When the unsaponifiable matter has been separated it should be examined as to its complete freedom from soap by burning a portion and testing for an alkaline ash. If this is detected the material must be dissolved again in petroleum and washed. It should then be crystallised from alcohol, the crystals examined for those forms that have been described as characteristic for cholesterol and the related alcohols. The iodine value should be taken and the acetate, benzoate or other derivatives prepared. With regard to the iodine value it should be noted that the theoretical number required by cholesterol is 68·4, and this value has been obtained for it by Herbig and Lewkowitsch, working with Hübl's solution. Marcusson, however, found that cholesterol gave a very much higher figure when Wijs' iodine solution was used (109) than it did with Waller's acid iodine and mercuric chloride solution. In this case the same cholesterol gave the value 29. This discrepancy has not been explained. It has been noticed by Mottram, and confirmed by Fourie and myself, that the "cholesterol" obtained from the liver of rabbits

[1] When ether is used Bömer recommends, in order to avoid delay from the formation of emulsions, that the following proportions should be closely adhered to: 100 grms. fat, saponified with 200 c.c. of a solution of 200 grms. KOH in 1 litre of 96 per cent. alcohol, is dissolved in 600 c.c. of water and shaken with 800 c.c. of ether, and then two or three times each with 400 c.c. of ether. The combined ethereal solutions are evaporated to dryness, and the residue heated again with 10 c.c. of the same alcoholic potash for a quarter of an hour. It is then dissolved in 30 c.c. of water and shaken twice with 100 c.c. of ether.

and pigs, when tested with Wijs' solution, gives high iodine values, even higher sometimes than that found by Marcusson (*cf.* Ubbelohde, *Handbuch der Fette*, vol. i., p. 216). This observation, also, has not been explained.

The identification of cholesterol, isocholesterol and the kindred alcohols depends upon the recognition of the properties of these substances and their derivatives (described above, p. 35 *seq.*). It need not therefore be entered into again here. The estimation of cholesterol otherwise than as "unsaponifiable matter," according to the method described previously, clearly cannot be carried out in the present condition of our technique.

The separation, isolation and identification of *other alcohols*—those of the aliphatic series—also present but few points for discussion. The separation is, in the first instance, effected, as in the case of other unsaponifiable matter ; only it must be remembered that in the case of esters of the highest alcohols and the highest fatty acids the solubility in alcohol is low, and saponification takes much longer than is the case with the common glycerides. Röhmann recommends heating in a pressure-bottle with a solution of baryta in methyl alcohol ; Kossel and Obermüller treatment with sodium ethylate, obtained by dissolving 5 grms. of metallic sodium in 100 c.c. of absolute alcohol.

Sometimes, if large amounts of higher aliphatic alcohols are present, these will separate out from the alcohol in which the saponification has been carried out; and in the case of beeswax, for instance, with the separating myricyl alcohol, the potassium salt of cerotic acid also comes out of solution. If these are filtered off they can be separated by means of ether. Small quantities of higher aliphatic alcohols will remain dissolved in the alcoholic soap solution, and these can be obtained in the usual way by shaking a solution of the soap in 50 per cent. alcohol with petroleum ether. When the alcohols are obtained free from soap they should be crystallised from alcohol and the melting point taken, the acetate prepared, and the acetyl value determined. If cholesterol or such alcohols are present, as shown by the reactions for these substances, they may be separated by the operation by which at the same time steps are taken for identifying the particular aliphatic alcohol present, that is to say, by heating the mixture with soda lime. This converts aliphatic alcohols into the corresponding fatty acids. Cholesterol, of course, yields no fatty acid when thus treated, and indeed is but little changed. Octadecyl alcohol heated in this way with four times its weight of soda lime to 270° to 280° C. for twenty hours yields stearic acid (Röhmann). If a mixture of aliphatic alcohols were used, the resulting acids could be separated according to the methods given for separating saturated fatty acids. Hell has worked out a method for determining the amount of higher aliphatic alcohols in beeswax by measuring the amount of hydrogen given off on oxidation of the alcohol with potash lime : $RCH_2OH \rightarrow RCOOH + 2H$.

The hydrocarbons which are present in some waxes can be separated from the alcohols after these latter have been oxidised in this way by shaking the mixture with petroleum ether and 50 per cent. alcohol.

3. *The Separation and Identification of Phospholipines.*

As was mentioned above, the description of the several phospholipines that have been obtained from different sources can best be taken together with the account of the methods by which they have been isolated from the fatty materials in which they have been found.

One of the earliest attempts to isolate such a substance was made by Couerbe in 1834, who obtained from the brain a substance ("cérébrote") which contained phosphorus, and was the forerunner of the protagones described later by others.

Some years later (1847) Gobley described the substance which he called lecithine, a component of the yolk of egg, in which he determined the presence of glycerophosphoric acid, fatty acids and nitrogen.

Without going more in detail into the history of the chemistry of these substances, which would be unprofitable, seeing that even at the present time there is so much that is still uncertain, it will be best to give a short account of the most satisfactory attempts to isolate pure substances from the various materials with which such attempts have been made, namely :—

1. Yolk of egg.
2. Brain and nerves.
3. Muscle.
4. Liver.
5. Kidney.
6. Blood.
7. Various vegetable seeds.

(1) *Yolk of Egg.*

Stern and Thierfelder dried the yolks of 100 eggs (887 grms.) spread out in a thin film on glass plates in front of a fan. In a few hours it was dry enough to be minced into fine pieces which were then dried *in vacuo* till they contained only about 1 per cent. of water. The dried yolk was shaken with 1½ litres of ether for some hours and then filtered on the pump, the filtrate concentrated *in vacuo* and precipitated with acetone, and the precipitate kneaded with fresh acetone. This treatment of the dried yolk with ether was repeated in all five times till finally the addition of acetone to the ether solution gave very little precipitate. All the operations were carried out in vessels from which air was as far as possible excluded and protected from the action of light, and the solvents were anhydrous and freshly distilled. These precautions were observed through all the stages of the preparation, and at no time were the substances heated except once in the case of the "diamino" fraction (*vide infra*), or when alcohol was to be evaporated, which was done *in vacuo* at body temperature, all other solvents being evaporated at the room temperature *in vacuo*.

The acetone precipitate alone was examined; the substances left in solution contained 0·45 grm. of phosphorus, but were not investigated. It was treated with ether and found not to dissolve at once to a clear solution; the ether quickly centrifuged deposited a white substance from which the clear ether was decanted. The clear ethereal solution was concentrated and treated with acetone, the precipitate dried *in vacuo*, and this whole operation repeated four times, as long as it was found possible to separate in this way any more of the white substance in the centrifugal machine.

This white substance, which will be referred to as the "diamino" fraction, and which is only slightly soluble in ether, was removed, though not quite completely. It was purified by suspension in ether and centrifuging the suspension; as it is not entirely insoluble in ether this necessarily involves loss. It was then warmed to 45° to 50° in alcohol, filtered from traces of protein, concentrated and treated with acetone. The precipitate, which was not smeary, was washed with acetone and ether, and dried. The yield of this substance, though necessarily in no sense quantitative, amounting to only 0·75 grm., indicates at any rate that it is present in considerably smaller quantities than those to be described.

The rest of the substances insoluble in acetone, dissolved in ether and without being much concentrated, were precipitated with acetone, the acetone precipitate at once kneaded with fresh acetone and dried *in vacuo*. This operation, the object of which was to remove fat and cholesterol, was repeated so long as the decanted acetone ether was found to contain on concentration any fat; some of this was fat containing phosphorus, and the amount so lost was reckoned to be about 3 to 4 grms. The acetone precipitate, after repeating this operation four times, weighed 22 grms.

The dry material was treated with alcohol: part dissolved and part did not. By means of the centrifuge a clear alcoholic solution,

referred to as the "lecithine" fraction, was separated from a syrupy material insoluble in alcohol but soluble in ether, which will be referred to as the "kephaline" fraction.

(1) This latter was dissolved in ether, concentrated and precipitated with alcohol: the precipitate rubbed with alcohol and then with acetone became powdery. It was then treated with ether and centrifuged, the clear ether solution was concentrated and precipitated with alcohol and again the precipitate rubbed with alchohol and acetone to a powder. Between 4 and 5 grms. of the dry powder was obtained which was insoluble in alcohol, but gave in ether after about a minute a clear solution.

(2) The alcoholic solution, "lecithine" fraction, concentrated and treated with acetone gave a viscid orange-coloured precipitate which could not be reduced to a powder. It dissolved in alcohol, but gave a precipitate on adding more alcohol so that it contained still some of the "kephaline" fraction. This was removed by centrifuging, concentrating, precipitating with acetone, drying and dissolving in alcohol; this process was repeated, finally after cooling the alcoholic solution down to 0°, altogether four times. Traces of the "diamino" fraction were still present, however, and could be removed by centrifuging the ethereal solution. At the end of this treatment about 4 grms. of the "lecithine" fraction was obtained, dissolving clear in either alcohol or ether.

The three fractions thus obtained differed very markedly in their physical properties. The orange-coloured "lecithine" fraction, which they do not consider to be yet a completely pure substance, when dried *in vacuo* becomes stiff, but cannot be powdered because it is so intensely hygroscopic.

The "kephaline" fraction obtained as a light yellow powder, showing a tendency to darken, is also hygroscopic but not to the degree to which the "lecithine" fraction is. When heated in a capillary tube drops appear between 100° and 110° C., but it does not strictly melt and decomposes between 140° and 150° C., giving off gas. The "diamino" fraction, on the other hand, is a pure white substance which does not change in colour when kept in an exhausted desiccator exposed to the light, is far less hygroscopic even than the "kephaline" fraction, and when heated in a capillary gradually becomes yellow and melts to a brownish red oil at 169° to 170° C. It crystallises from alcohol in rosettes of straight or sometimes curved needles.

On analysis the following results were obtained:—

	"Lecithine" Fraction.	"Kephaline" Fraction.			"Diamino" Fraction.
		(1)	(2)	(3)	
C	64·63	65·66	59·68	59·5	59·48
H	10·96	11·54	9·74	9·8	9·42
N	2·08	1·37	1·57	1·75	3·47
P	3·97	3·96	3·64	3·83	3·84
Cl	present	0·31	—	—	present
Ca	present	1·03	—	—	trace
I. Val	48·7	70·4	—	—	34·3
P : N	$\frac{1}{1·16}$	$\frac{1}{0·77}$	$\frac{1}{0·96}$	$\frac{1}{1·01}$	$\frac{1}{1·9}$

The "kephaline" fraction, (2), was obtained in precisely the same way as (1) but from a commercial preparation of dried yolk; the differences in the analytical results cannot be accounted for, but they result in a close similarity of the substance analysed, (2), to the kephaline obtained by Koch from the brain, the figures for which are given in (3); and also the kephaline described by Thudichum in the brain. This kephaline resembles the "kephaline" fraction from yolk also in its insolubility in alcohol, and in the fact that it can be obtained as a powder. Koch also describes a kephaline obtained from yolk of egg.

Erlandsen also notes that the "lecithine" obtained from egg yolk with alcohol contains more nitrogen than the usual formula for lecithine requires, whereas the "lecithine" fraction of the ether extract (acetone precipitate soluble in cold alcohol) contained only compounds with an equal number of nitrogen and phosphorus atoms.

(2) *Brain and Nerves.*

The examination and identification of the compounds of fatty acids in nerve tissues is a subject on which there is very little to be said that is definite and final, though very much work has been done that has not this character. The chemical study of nervous tissues has been mainly concerned with the compounds of fatty acids found in them, but nevertheless these compounds remain some of the most obscure and ill-defined substances with which physiology has to deal. Taking a broad general view of this study it may be said to be divisible into two periods: a period in which the belief prevailed that the characteristic constituent of nerve tissues was protagone, a substance of great complexity obtained in apparently crystalline form by the simplest means from the white sheath of nerve fibres. And secondly, a period in which this belief has been attacked and shaken from its foundations.

Protagone was first prepared by Liebreich in 1865 who after a preliminary treatment with ether heated the brain with 85 per cent. alcohol at 45° C. for twelve hours, and allowed the hot filtered alcoholic extract to cool slowly. The precipitate that formed was washed with cold ether and "recrystallised" from warm alcohol. It has been prepared by others since, using methods which differ from Liebreich's only in unessentials. Protagone therefore is a substance that is only slightly soluble in ether, soluble in hot but not in cold alcohol. When it has been pointed out that in the substance so obtained a number of different substances can be shown to be present, the answer has been that these are all parts of the protagone molecule, and if they are isolated from a preparation of protagone that implies that the protagone has been decomposed. Protagone therefore is supposed to be a single complex combination of all the chemical compounds that can be demonstrated in nerve tissues, a combination that is in the same class with "protoplasm" and as little capable of chemical definition.

But the evidence for the chemical entity of protagone is of the slenderest. By recrystallisation its composition is altered, especially if this be done fractionally, the different fractions then having different amounts of phosphorus ranging from 0·1 to 2·9 in an experiment of Thudichum's. The same result was obtained by Rosenheim and Tebb who also showed that the rotatory action of the different fractions upon polarised light was different, and that the different fractions yielded on hydrolysis different amounts of galactose. The fact that from a tissue like the brain by the simple procedure followed in preparing protagone a product of fairly constant composition should be obtained is no proof that this is a single substance and not a mixture. And as a matter of fact in order to isolate from this product what are called its cleavage products it is not necessary to use aqueous or methyl alcoholic solutions of baryta as was done by Kossel and Freytag and others, nor any active hydrolytic reagents: simple selective solvents, a mixture of methyl alcohol and chloroform (Thierfelder) or pyridine (Rosenheim and Tebb) suffice to separate the ingredients of this mixture.

Thudichum devised methods for the separation and identification of many substances from the brain, which he worked at for years, without, however, furnishing convincing proof that the operations involved were without influence upon the chemical composition of the substances submitted to them. It is not necessary therefore to follow in detail all the intricate subdivisions of his classification of the "phosphatides". It is sufficient to note that he recognised the presence in the brain of more than one substance like lecithine containing one atom of nitrogen for one of phosphorus, and, in addition to these, substances containing two atoms of nitrogen for one of phosphorus, possibly others containing two atoms of phosphorus and two of nitrogen.

His methods consisted principally in: (1) the precipitation of extracts of the brain in alcohol with alcoholic cadmium chloride solution, and the separation of the components of this precipitate by means of solvents; (2) precipitation of extracts of the brain in alcohol with alcoholic lead acetate and ammonia.

Thus the brain extracted with hot alcohol gives a solution which on cooling deposits a "white substance". A solution of this deposit in ether, treated with absolute alcohol, gives a precipitate consisting mostly of kephaline. The filtered alcohol ether and the original alcoholic mother liquor, from which the white substance was deposited, treated hot with alcoholic lead acetate and ammonia, can be freed from certain substances, principally kephaline, and the filtrate, after driving off ammonia and ether, treated with excess of cadmium chloride in alcoholic solution. The cadmium precipitate, washed with alcohol, dried and powdered, is exhausted with hot ether, in which the cadmium compounds do not dissolve, and then, freed from ether, is treated with benzene. This at first dissolves only such traces of the cadmium compound of kephaline as may be present, which are removed, and then the mixture is heated with benzene to its boiling point. At this temperature much of the

cadmium precipitate dissolves; for the lecithine cadmium compound is "dehydrated," and so becomes soluble even in cold benzene. If the boiling benzene solution is filtered the cadmium compound of amidomyeline is left on the filter, that of paramyeline separates from the filtrate as it cools, and that of lecithine remains in solution and can be thrown out by the addition of alcohol. This last is purified by crystallisation from boiling alcohol, in which it dissolves.

The cadmium chloride compound of lecithine is treated in hot alcohol with sulphuretted hydrogen; the solution filtered gives on cooling crystallised hydrochloride of lecithin, and the mother liquor treated with Millon's base—$O\begin{smallmatrix}NH_2Hg_2O\\NH_2Hg_2O\end{smallmatrix}$—so as to remove hydrochloric acid, can be used for the preparation of free lecithine. Free lecithine was also prepared by suspending the cadmium compound in water and removing the salt by dialysis.

Kephaline is obtained, as indicated above, as a precipitate with absolute alcohol from a concentrated ethereal solution of the "white substance," thrown down when the original alcoholic extract cools, and also by precipitating alcoholic solutions in which it is present mixed with other substances by means of alcoholic solution of lead acetate and ammonia. In the first case it is washed with ether, then emulsified with water and filtered through paper under pressure, and from the filtrate precipitated with hydrochloric acid. The precipitate is freed from water by alcohol dissolved in ether and reprecipitated with alcohol and dried. If precipitated with lead the lead compound dissolves readily in ether and can be thus separated from the lead compound of "myeline". The cadmium chloride compound of kephaline dissolves in ether easily and also in benzene. Several modifications and products of the spontaneous oxidation of kephaline are referred to by Thudichum.

Myeline is obtained as a lead salt insoluble in ether and in alcohol, or in another modification soluble in hot alcohol but not in cold. Sphingomyeline is the principal substance containing phosphorus that is present in protagone; that is to say, it is not removed from the brain by ether, in which it is almost insoluble, but dissolves in hot alcohol and separates from its solution on cooling. Thudichum separated it from the other constituents of protagone by means of cadmium chloride, the cadmium compound being less soluble in alcohol than the cerebrines.

Sphingomyeline and the substance amidomyeline mentioned above are instances of Thudichum's phosphatides, containing two atoms of nitrogen for one of phosphorus, the other substances mentioned in the previous paragraphs—lecithine, kephaline, myeline and paramyeline—all containing only one atom of nitrogen to one of phosphorus.

Thudichum's account of his work is more circumstantial in the conclusions and inferences drawn than in the data from which they depend; and the legitimacy of some of his methods has been called in question by Erlandsen. First of all, with regard to the use of cadmium chloride as a precipitant for such substances as these,

Erlandsen found that the lecithine of the heart, three different preparations of which were identical in composition, was not precipitated quantitatively by cadmium chloride, one-third of the lecithine being held up in solution. Then the amount of cadmium chloride in the precipitate was different from that given by Thudichum—1·5 not 1 molecule of cadmium chloride for one atom of nitrogen and one of phosphorus; and, moreover, analysis of the cadmium compound showed that a change had occurred in the organic molecule, the figures obtained requiring the formula $C_{37}H_{69}NPO_8$ instead of $C_{43}H_{80}NPO_9$, which was indicated by the analysis of the lecithine from which the cadmium precipitate was obtained. This suggests that the precipitation of such compounds with metallic salts is not necessarily unaccompanied by chemical changes, and raises questions as to the nature of the products obtained. When, again, he tested Thudichum's method of separating cadmium chloride precipitates by means of their solubility in ether and benzene he obtained results which make it desirable that Thudichum's work should be tested by critical experimentation before his conclusions are adopted *en masse*. A cadmium chloride precipitate, 54 grms., showing the ratio P : N : $CdCl_2$ = 1 : 2 : 2, was extracted continuously for fourteen days with ether, and 15 grms. recovered from the ethereal solution. The portion insoluble in ether weighed only 34 grms. instead of 39 grms., which is a suspiciously large difference. This portion was then treated with benzene exactly as done by Thudichum, with again considerable loss of substance—about 10 per cent. In this way the following fractions were obtained, and on analysis showed the figures given :—

	Calculated formula,	P : N : $CdCl_2$.
I. Original compound - -	$C_{40}H_{75}N_2PO_{12}$	1 : 2 : 2
I. Soluble in ether - -	$C_{41}H_{73}N_2PO_{10}$	1 : 2 : 1·7
II. Insoluble in ether - - -	$C_{35}H_{66}N_2PO_{12}$	1 : 2 : 2
II. (*a*) Soluble in cold benzene -	$C_{34}H_{62}N_2PO_{10}$	1 : 2 : 1·8
(*b*) Soluble in hot benzene -		1 : 2
(*c*) Insoluble in hot benzene -	$C_{31}H_{66}N_2PO_{12}$	1 : 2 : 2·3

In all these fractions, therefore, the ratio of P : N was 1 : 2, and the method which in the case of Thudichum's preparations from the brain seemed to promise to serve as a method for separating substances in which the P : N ratio is 1 from those in which it is 1 : 2 is shown to have no such significance necessarily. The results also suggest that it is necessary to prove that prolonged treatment with boiling ether and also boiling with benzene can be tolerated by such sensitive compounds before all the products obtained, named and described by Thudichum can be recognised as actual native components of the brain.

In many other particulars Thudichum's technique is open to criticism, for instance, treatment with hydrogen sulphide in boiling alcohol for the removal of metals, boiling in alcohol with ammonia and lead acetate, prolonged dialysis of aqueous suspensions of lecithine to remove cadmium chloride, and indiscriminate evaporation of solvents on hot water-baths at all stages of the procedure.

Nevertheless, Thudichum was the first to slay the oft-slain "protagone," was the first too to show that besides lecithine, and probably mixed with it in most preparations of this substance that have been analysed, there are other compounds of fatty acids containing phosphorus and nitrogen, and not necessarily in the same atomic proportions as in the traditional lecithine.

Koch has found a kephaline in the brain, possibly the same as Thudichum's in composition, and resembling it in its insolubility in alcohol and in giving a precipitate with lead acetate and ammonia. The main difference between kephaline and lecithine, according to Koch, is that kephaline contains no choline, but the corresponding monomethylamine base instead. He obtains kephaline from sheep's brains by dehydrating them with an equal weight of acetone, and, after removing the acetone, extracting with ether and precipitating from the ether solution, after it has undergone spontaneous concentration in the air and has been filtered, the kephaline by means of alcohol. After further treatment with ether the kephaline is recrystallised from ethyl acetate. The prolonged exposure to the air during the preparation may possibly account for the large amount of oxygen in the formula given, $C_{42}H_{82}NPO_{13}$. Lecithine has only nine atoms of oxygen.

Koch finds that the one methyl group attached to nitrogen in kephaline is removed at 240° C., whereas in lecithine, while one comes off at 240° C., the other two are not removed till the temperature 300° C. is reached. Koch has devised a method for estimating kephaline and lecithine separately, which consists in determining the phosphorus in the precipitate on the one hand, obtained with lead acetate and ammonia from the substances extracted by alcohol and ether from nervous and other tissues, and in the filtrate from this precipitate on the other hand. In the corpus callosum he finds thus nearly as much kephaline as lecithine. He has applied the same method to other tissues and materials, and finds that egg "lecithine" is really two-thirds of it kephaline.

Rosenheim and Tebb have given a short preliminary account of a method for obtaining sphingomyeline from the brain, but the exact composition of this substance, and how nearly it corresponds to the substance described by Thudichum, is not yet apparent from the few data published at present. The brain substance dried with plaster of Paris powdered and freed from cholesterol with cold acetone is extracted with boiling acetone, from which on cooling "protagone" separates. This is heated with pyridine, and from the pyridine sphingomyeline crystallises out in anisotropic globules. It is purified by fractional precipitation from a solution in alcohol and chloroform by means of acetone. The final product is white, not at all hygroscopic nor liable to spontaneous oxidation in air, and contains two atoms of nitrogen for one of phosphorus and 4 per cent. of the latter. Like Thudichum's substance, it contains no glycerol; it contains fatty acid radicals and choline.

(3) *Muscle.*

Erlandsen's examination of the substances like lecithine in the heart and voluntary muscles of the ox is in many respects one of the most satisfactory contributions to our knowledge of the phospholipines that have yet been made. Erlandsen's procedure consists essentially in the first place of drying the minced material rapidly in a warm room before a fan; in twelve hours he found that muscle would lose in this way 70 to 75 per cent. of its weight. The mass, cut up into small pieces, was then further dried in a vacuum apparatus at 40° C. for five to six hours, when it was in a condition in which it could be ground to a powder in a mill. This powder was extracted first with ether and then with alcohol, and great stress is laid by him on the importance of observing this order. The extraction was carried out in stoppered vessels, frequently shaken, at the room temperature. The ether was changed daily for four or five days, afterwards four or five times more at longer intervals; small quantities of extract were thus removed, while the larger quantities obtained in the early extractions were being examined.

After complete exhaustion with ether the mass was pressed, and the last traces of adherent ether having been removed by exposure to the air, the extraction with 90 to 96 per cent. alcohol was carried out similarly, in closed glass vessels. The alcohol was changed and pressed out five or six times at intervals of twenty-four to forty-eight hours, the vessel having been kept at a temperature of 40° to 45° C. for part of each such period.

All the ether and alcohol extracts were concentrated as soon as obtained *in vacuo* at a low temperature, and the syrupy residues kept in carbonic acid in the dark.

The examination of these extracts showed that the ether removed substances which were different from those removed by alcohol.

The ether extract was dried and taken up in dry pure ether, in which it mostly dissolved, the insoluble remainder (*a*) being largely inorganic.

The ethereal solution was separated into two parts: one soluble in acetone and one insoluble. The former, consisting mostly of simple glycerides, nevertheless contained 0·3 per cent. of phosphorus which was present in the form of some phospholipine containing equimolecular amounts of phosphorus and nitrogen.

The acetone precipitate was divided by means of alcohol into a substance (*c*) soluble in cold absolute alcohol; a substance (*b* ii.) soluble in warm absolute alcohol; and a substance (*b* i.) insoluble in alcohol even on warming.

The first of these (*c*) he calls lecithine. It is soluble in alcohol, ether, chloroform, acetic ether and petroleum ether. It is at any rate very slightly, if at all, soluble in acetone at the ordinary temperature; with water it slowly gives an emulsion of a slimy character, as is usually found with substances described as lecithine. It

gives Pettenkofer's reaction and melts about 60° C., but not sharply, and is not perceptibly decomposed till 110° C. is reached. Dissolved in alcohol it gives a precipitate with an alcoholic cadmium chloride solution, which, after washing with alcohol, is soluble in benzene and at first in ether, though after being dried it is insoluble in ether even when heated, though on warming it still dissolves in benzene. The alcoholic solution is also precipitated with alcoholic platinum chloride solution.

The iodine value of the lecithine itself was 100·4 when freshly prepared, while in a sample kept for some months in an unexhausted desiccator it fell to 29.

When saponified with baryta fatty acids were obtained, and from their amount and the molecular weight of the lecithine and the amount of alkali used in titrating the fatty acids it was found that the molecule contained two fatty acid radicals.

The fatty acids obtained from one preparation amounted to 65·15 per cent., in another 70·50 per cent. of the lecithine; a sample of the acids analysed gave figures agreeing with the formula $C_{18}H_{32}O_2$.

The other substances found after saponification of the lecithine were obtained from the aqueous filtrate after removing the barium soaps. Excess of barium was removed with carbonic acid and the fluid evaporated to dryness. The residue taken up in absolute alcohol gave a yellow precipitate with alcoholic platinum chloride, which, crystallised from water, contained the amount of platinum present in the double salt of choline and platinum chloride. The amount of this salt that was obtained, it is true, was only 42 per cent. of the theoretical yield, if all the nitrogen were originally present in the molecule of the lecithine as choline. In addition to choline the residue contained substances or a substance insoluble in alcohol. Crystals were obtained from an aqueous solution which contained barium and phosphorus, and gave the acrolein reaction for glycerol. But a quantitative examination showed that it was not pure glycerophosphate of barium.

The elementary analysis of the lecithine itself gave figures from which the formula $C_{43}H_{80}NPO_9$ was calculated, the conventional formula for distearyl-lecithine being $C_{44}H_{90}NPO_9$.

The fraction *b* resembled kephaline in being insoluble in alcohol, cold or hot, but not in other respects, and is therefore given the name *cuorine*. It was when dry a hard, yellowish-brown, transparent substance too hygroscopic to powder. It dissolved readily in ether, chloroform, carbon bisulphide or petroleum ether, less readily in benzene, and in warm acetic acid, acetic ether and amyl alcohol, from which solvents it separates on cooling; it was insoluble in ethyl- or methyl-alcohol and acetone, even at their boiling points. In water it swells up and gradually forms a sort of turbid emulsion or solution. The addition of alkalies but not their carbonates causes this turbidity to disappear. It dissolves in concentrated sulphuric acid and gives Pettenkofer's reaction. An ethereal solution with alcoholic platinum chloride gives a yellow precipitate only with large quantities of the reagent and much alcohol.

The ethereal solution also gives a precipitate with alcoholic cadmium chloride, which is colourless. The substance readily undergoes spontaneous oxidation, even in ethereal solution in a full stoppered bottle kept in the dark, but much more markedly in an unevacuated desiccator. A preparation which when fresh gave on analysis the empirical formula $C_{71}H_{125}NP_2O_{21}$, gave after being thus kept for some months the formula $C_{71}H_{125}NP_2O_{30}$; similarly a preparation gained 9 per cent. in weight during a month over sulphuric acid. At the same time the iodine value fell, in one case from 101 to 22, and it ceased to be soluble in ether and other solvents, but became soluble in water, giving a perfectly clear solution.

On saponification it yields fatty acids, each molecule three molecules of these, amounting to 64 per cent. of the weight of the cuorine, and having the mean empirical formula on analysis, $C_{19}H_{34}O_2$, and the mean iodine value 130. Glycerophosphoric acid could also be demonstrated to have been split off during saponification, and a base which behaved differently from choline in many respects, giving a platinum salt containing 37·3 instead of 31·6 per cent. of platinum, but nevertheless, on heating, the smell of trimethylamine.

The substance soluble in warm but not cold alcohol was present in too small amount to be definitely characterised. But from the ether extract two substances were obtained, a lecithine and cuorine, both of which were studied as fully as any of this class of substances ever have been.

The substances extracted from the heart muscle by alcohol after complete exhaustion with ether were similarly divided up by the use of different solvents. They were dried, and by means of absolute alcohol insoluble substances, mostly inorganic, were removed. A considerable part of what dissolved in absolute alcohol was insoluble in absolute ether, but this was a mixture of various substances not identified but containing altogether only 2 or 3 per cent. of fatty acids. The part soluble in ether was divided into two by means of acetone; the part that was soluble appeared to contain some phospholipine, as it gave a precipitate with cadmium chloride containing phosphorus and nitrogen, but it was in comparatively small amount. The acetone precipitate contained much the largest part of the phospholipines. It was freed by means of absolute alcohol from an insoluble substance or mixture that behaved qualitatively in several respects like jecorine, and it was then treated with cadmium chloride in alcoholic solution, and the precipitate washed, dried and analysed. The empirical formula calculated from double analyses of each of two preparations was $C_{40}H_{75}N_2PO_{12}.2CdCl_2$, and leads therefore to the conclusion that a substance containing two atoms of nitrogen and one of phosphorus exists in cardiac muscle, in this respect resembling the amidomyeline and sphingomyeline of the nerve tissues found by Thudichum. When the attempt was made to separate the cadmium chloride precipitate into fractions soluble in ether, or soluble in hot or cold benzene as done by Thudichum, fractions were obtained which were all alike in con-

taining two atoms of nitrogen for one of phosphorus, but differed in other respects in their elementary composition. And the attempt, too, to recover a cadmium and chlorine free preparation from the cadmium chloride precipitate, though successful so far as the removal of cadmium and chlorine was concerned, resulted with considerable loss in a product which had certainly been changed in the process, as the relation of two atoms of nitrogen for one of phosphorus was lost.

On saponification of the cadmium chloride precipitate itself, however, it was possible to show the presence of fatty acid, apparently an oxy-acid of high molecular weight, probably $C_{22}H_{40}O_4$, of which each molecule of cadmium compound gave only one molecule, and about 45 per cent. of its weight, and in addition to this, glycerophosphoric acid and a base that seemed to be a trimethylamine derivative but not choline.

Erlandsen made preparations of the phospholipines of voluntary muscles (ox) in exactly the same way. The amount of phospholipines was much smaller, the ether extract being only one-third of the amount obtained from the heart, though there was little difference between the amounts extracted by alcohol from the two tissues. The ether extract on fractionation was principally found in the lecithine fraction, the cuorine fraction being represented by only 1 grm. from 10 kgrms. of muscle. The lecithine gave on analysis figures that point to the same formula as the lecithine from the heart, $C_{43}H_{80}NPO_9$, with ten atoms of hydrogen less than are required by "distearyl-lecithine".

The alcoholic extract contained considerably less phospholipines than the similar extract of heart muscle, less than half the amount. Here, too, the cadmium chloride precipitate contained two atoms of nitrogen for one of phosphorus, though the empirical formula calculated from the analyses works out somewhat differently in other respects.

A comparison therefore of the phospholipines in these two types of muscle shows that—

(1) The heart contains from two to three times as much of these substances as voluntary muscle.

(2) Both contain lecithine, N : P = 1 : 1.

(3) The heart contains cuorine, N : P = 1 : 2, in very much greater amount than ordinary muscle, which can contain only very little of this.

(4) They both contain a phospholipine in which N : P = 2 : 1, though probably not the same substance. These two phospholipines resemble one another in the fact that, though soluble in ether, they are not in the first instance extracted by this solvent. They are removed from the dry tissue only when it is treated with alcohol, but when once they are extracted they dissolve in ether.

(5) Nothing of the nature of protagone was found in either kind of muscle;

(6) And only traces of anything resembling jecorine.

(4) *The Liver.*

Drechsel described a substance containing nitrogen, phosphorus and sulphur which he obtained from the liver of the horse and porpoise, and called jecorine.

He obtained it by extracting the minced organ with alcohol, evaporating the alcohol at 40° to 50° C., treating the residue with absolute alcohol and what would not dissolve in this with ether. The ethereal solution was precipitated many times with absolute alcohol, and the precipitate dried to a light, porous, yellow powder that was intensely hygroscopic.

The dry substance was insoluble in dry ether but dissolved in ether when wet; it dissolved in water slowly, at first swelling up to a slimy mass but finally giving a clear solution.

Treated with alkali and heated, soaps were formed which set to a jelly on cooling. It reduced copper strongly and gave a remarkable red colour when heated with silver and ammonia.

On analysis it was found to contain nearly two atoms of nitrogen for one of phosphorus.

This substance has since been studied by Baldi, Manasse, Baskoff and others. Manasse studied the cleavage products and isolated choline, fatty acids, glycerophosphoric acid, and a phenylosazone melting at 203° to 204° C. Baskoff determined the amount of sugar in a number of different preparations and found remarkably constant figures about 14·5 per cent. In his preparations, too, there were two atoms of nitrogen for one of phosphorus, and about 40 per cent. of higher fatty acids were set free on saponification.

Jecorine has never been isolated in such a way as to establish finally its chemical character; but the contention that it is a mixture or combination of lecithine and glucose such as has been artificially prepared by Bing, Mayer and others is confuted by Baskoff's analyses of the preparations made by him. The ratio N : P and the constant amount of sugar found show that jecorine is not to be disposed of by pointing to the resemblance to it in certain respects of an artificial lecithine glucose.

Baskoff obtained other preparations of phospholipines from the alcohol used for washing or precipitating jecorine. The alcohol was evaporated, the residue dissolved in ether and repeatedly precipitated with acetone. These preparations also contained two atoms of nitrogen for one of phosphorus, contained 62·5 per cent. of fatty acids and two such radicals in the molecule.

On the other hand, when he treated the liver in the way in which Erlandsen dealt with the heart he was unable to find jecorine among the products, though he was also not able to find exactly the substances which Erlandsen isolated from the heart.

In the bile it is usually said that "lecithine" occurs, which means no more than that the ether extract contains phosphorus and nitrogen. Thudichum expressly says that there is no true lecithine in ox bile and that he found in it a "phosphatide" in which the

P : N ratio was 1 : 4. Hammarsten in the bile of the polar bear found that platinum chloride gave a precipitate in an alcoholic solution of the substances precipitated by acetone but soluble in ether, and that this precipitate contained phosphorus, nitrogen and platinum in the proportions required by dipalmityl lecithine. He also obtained the cleavage products of lecithine, choline, glycerophosphoric acid and fatty acids. But in addition to this evidence for the presence of lecithine he also found that cadmium chloride gave precipitates containing more nitrogen than lecithine: in one preparation $P : N : CdCl_2 = 1 : 2 : 2$, in another = 1 : 3 : 2. He does not consider that he had pure substances, but thinks the evidence justifies the conclusion that other bodies resembling but not identical with lecithine are present in the bile of this animal.

(5) *The Kidney.*

Dunham treated kidneys of oxen in the same way as Cramer treated the brain in preparing "protagone," *i.e.*, the minced material was heated with 5 per cent. sodium sulphate solution at 85° to 90° C., and the coagulum extracted first with 95 per cent. then with 85 per cent. alcohol at its boiling point. The alcohol filtered hot and cooled to 0° C. gave a precipitate which was extracted with ether, and the insoluble part crystallised out of chloroform solution. This "protagone" contained twice as much phosphorus, 50 per cent. more nitrogen than Cramer's preparation from the brain. It clearly contained compounds similar to those in protagone from the brain, because on hydrolysis fatty acids and a reducing substance were obtained, and evidence for methyl groups attached to nitrogen was forthcoming. But though the phospholipines and other substances of which it was composed were not isolated, a remarkable fatty acid was found to be liberated on saponification and its properties studied, a carnaubic acid, $C_{24}H_{48}O_2$ (*vide supra*, p. 12).

The "protagone" bodies described by histologists in "large white" kidneys appear to be cholesterol esters (Panzer).

(6) *Blood.*

Lecithine was obtained from the blood by Hoppe-Seyler, and its cleavage products identified by Manasse.

Wooldridge extracted the stromata of red blood corpuscles with 85 per cent. alcohol at 45° C., evaporated the cold filtered solution at the same temperature, took up the residue in absolute alcohol and evaporated the solution, and found the residue had the physical properties ascribed to lecithine. Its alcoholic solution gave a precipitate with alcoholic platinum chloride solution in which he found the ratio P : Pt : Cl :: 2 : 1 : 6, which is the same as that in the platinum compound of lecithine described by Strecker and by Thudichum.

In Bang and Forssman's study of the lysinogenetic substance in red blood corpuscles they found that the active body was extracted

by ether, insoluble in acetone, soluble in hot benzene, but in other respects did not behave as if it were a phospholipine; it was, for instance, insoluble in hot alcohol, and after treatment with acetone no longer soluble in ether. Incidentally they refer to their having demonstrated the presence of "a kephaline, a sphingomyeline and an amidomyeline" in blood, the kephaline differing from that obtained by Thudichum from the brain. Thudichum obtained amidomyeline from the blood.

It would appear therefore that besides lecithine (N : P = 1) some other body or bodies in which N : P = 2 may be isolated from the blood as well as from the brain, muscles and liver.

(7) *Vegetable Materials.*

Schulze and workers in Schulze's laboratory have described lecithine preparations obtained from various seeds and other vegetable materials by the following method: The finely ground seeds are exhausted with ether to remove fats and then extracted with alcohol at 50° C. The alcoholic solution is evaporated and the residue taken up in ether and repeatedly shaken with water, emulsions being broken up by the addition of solid sodium chloride. The clear ether solution is evaporated, and the residue is the raw material which has been studied. It contains, in the case of Vicia sativa, Lupinus luteus and Pinus cimbra about 3·6 per cent. of phosphorus and the ratio P : N = 1. But in the case of cereal seeds—rye, barley, wheat or oats—considerably less phosphorus is found, generally less than 2 per cent. This is in part accounted for by the fact that almost all the vegetable "lecithines" examined yield carbohydrates on hydrolysis. The amount varies, and may be as high as 16 per cent., reckoned as glucose from the amount of copper reduced. But the low percentage of phosphorus in "lecithine" preparations from cereal seeds is not accounted for entirely in this way; and the fact that the P : N ratio is less than one shows that some other phospholipine is present besides "lecithine" in the usual sense.

Winterstein and Hiestand, in Schulze's laboratory, have specially studied the phospholipine preparations from cereal seeds. The carbohydrate is more firmly united than in artificial lecithine sugar compounds. Galactose was identified, but glucose was shown to be present as well, and small quantities of pentose and methyl-pentose. The preparations were soluble in ether, chloroform, benzene or carbon tetrachloride, less so in alcohol or ligroin, and hardly soluble in acetone or methyl-acetate. This last solvent was found to be the most efficient agent for purifying the crude products and freeing them from fats and cholesterol. A considerable part—nearly half—was precipitated by lead acetate, suggesting the presence of a kephaline, and the high platinum content of the platinum double salt of the base set free on hydrolysis also suggests the presence of a compound of some base containing more nitrogen than choline. Estimations of the methyl groups, carried out, as done by Koch, by the Herzig-Meyer process, give support to this conclusion too.

Besides the cereal seeds, pollen of certain conifers and of an alder, certain funguses and the leaves of the horse-chestnut were examined and gave similar results.

To speak, therefore, of the vegetable lecithines is to use the term "lecithine" in a generic sense. The cleavage products of lecithine may be obtained from these substances it is true, and in one or two cases the amount of phosphorus and the ratio of phosphorus to nitrogen agree with the hypothesis that the substance present is lecithine, but in other cases they do not; and in no case were complete analyses carried out, because it was not supposed that the preparations represented pure substances.

CHAPTER IV.

THE PHYSIOLOGY OF FATS.

A. THE BIOCHEMICAL SYNTHESIS OF FATS AND HIGHER FATTY ACIDS.
B. THE PHYSIOLOGICAL OXIDATION OF FATS.
C. THE ROLE OF FATS IN VITAL PHENOMENA.

A. THE BIOCHEMICAL SYNTHESIS OF FATS AND HIGHER FATTY ACIDS.

THE fats that are found in animals are to a large extent derived from the fats contained in the food that they take. In each animal species there may be a certain mean composition characteristic of the fat of normal individuals of that species; for instance, the composition of beef suet, of mutton tallow, of lard and of goose fat is, broadly speaking, constant, and each of these kinds of fat differs from each of the others in a way that is characteristic of the species from which it is obtained. But this constancy does not depend probably so much upon the specific synthetic powers of the species as upon the nature of its food, the fats that this contains, and the way in which the fats are absorbed. Because, as is well known, the fat of an individual animal may be altered by feeding it upon fats different from those that are usually contained in its food. There is much evidence to show that whatever fat is absorbed from the intestine can be found unaltered in the connective tissue. The fat of an animal fed on colza oil contains erucin, that of one fed on linseed oil contains linolein. This is true, too, of man, erucin having been demonstrated in the chyle of a patient to whom erucic acid was administered. Even iodipin, fat or oil in which the unsaturated acids have been treated with halogens, can be absorbed and deposited without loss of its chlorine and iodine.

A large part, therefore, of the fats found in animals has been built up by vegetable organisms. And the synthesis of higher fatty acids is a problem that must be dealt with more particularly in connection with the physiology of plants.

There is, however, much evidence that the synthesis of higher fatty acids can be effected by animals also. The proof of this synthesis in the pig, and that the material used for it is carbohydrate, was furnished by the experiments of Lawes and Gilbert more than fifty years ago, and has been strengthened, if that was necessary, by many other experimenters since that time and extended to other species of animals.

Even in plants it is probable, though the proof is not so clear as in the experiments on animals, that the fatty acids are derived from substances that are formed by the breaking up of carbohydrates. Fats and oils occur in plants principally in the ripe seeds, nuts, or fruits. These parts before they ripen contain carbohydrates but not oil, and it is supposed that these carbohydrates, supplemented by those brought up from other parts in the sap, are transformed into oil during the ripening process. Sugar is found in the sap and oil or fatty acid is not.

The synthesis of fatty acids from carbohydrates has been proved to occur at any rate in animals, and the chemical changes involved are fascinating in their obscurity. But whatever the exact nature of these changes may be, it is obvious that they must be broadly of two kinds; the alcoholic hydroxyl groups that are characteristic of carbohydrates must be reduced, and condensations must occur in order that the long chains of carbon atoms may be formed of which the higher fatty acids are composed.

Now there is a group of changes which carbohydrates are liable to undergo in which it would appear that an alcoholic hydroxyl is reduced and its oxygen transferred to another carbon atom. This is at least the most probable interpretation of the formation of lactic acid and the saccharinic acids from sugar. In the case of lactic acid, which is so readily formed from sugar not only by the action of weak solutions of the alkalis, but also biochemically by bacteria, and in the animal body, it seems probable that the first step is the *disjointing of the carbohydrate chain* at the γ carbon atom by the change that is common in secondary alcohol groups in other cases; just as, for instance, the α-hydroxy acids split into an aldehyde and formic acid or its derivatives, leucic acid, *e.g.*, into isovaleraldehyde and formic acid, so glucose would by the same change split into two molecules of glyceric aldehyde:—

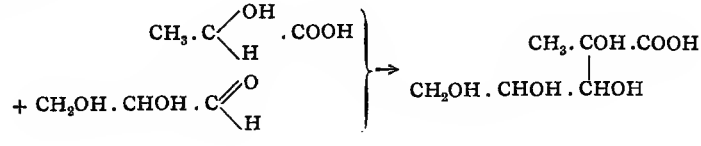
$$CH_2OH.CHOH.CHO \mid H.CHOH.CHOH.COH$$
$$\rightarrow 2CH_2OH.CHOH.COH$$

The next step then appears to be *the reduction of the β-alcoholic hydroxyl and the transfer of its oxygen to the terminal carbon atom* converting the aldehyde into an acid, in this case lactic acid:—

$$CH_2OH.CHOH.COH \rightarrow CH_3.CHOH.COOH$$

The evidence adduced by Nef for this interpretation is that the same treatment with weak alkalis leads to the formation of lactic acid from glyceric aldehyde and from glucose itself.

Kiliani explained the formation of Peligot's saccharinic acid from glucose under the influence of calcium hydroxide by supposing that a molecule of glyceric aldehyde then condenses with a molecule of lactic acid—



Windaus points out that the other saccharins may be formed in essentially the same way, for instance, parasaccharin by the carbohydrate chain being disjointed at the β-carbon atom so as to yield tetrose and glycollic aldehyde, and isosaccharin by disjointing at the α-carbon atom so as to yield pentose and formaldehyde. The subsequent reduction of the β-alcoholic hydroxyl in the tetrose or pentose and transfer of its oxygen to the terminal carbon atom of the aldehyde group and condensation of the resulting α-hydroxy acid with the glycollic or formic aldehyde would account for the formation of parasaccharin in the first case—

$$\begin{array}{l} CH_2OH.CH_2.COH.COOH \\ \quad\quad\quad\quad\quad\quad | \\ \quad\quad\quad\quad\quad CH_2OH.CHOH \end{array}$$

and of isosaccharin in the other—

$$\begin{array}{l} CH_2OH.CHOH.CH_2.COH.COOH \\ \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad | \\ \quad\quad\quad\quad\quad\quad\quad\quad\quad CH_2OH \end{array}$$

In all these changes to which the sugar is liable the reduction of the β-hydroxyl group of the β-hydroxy aldehyde appears to accompany the oxidation of the aldehyde group to the acid group: and one of these changes, that resulting in the formation of lactic acid, is a biochemical reaction.

Now Pasteur observed a remarkable reaction carried out by the bacteria that cause butyric fermentation of sugar, in which a fatty acid of the same series as stearic acid is formed from sugar together with carbonic acid and hydrogen. This same butyric acid was produced by the organism when lactic acid was given instead of sugar. The chain of four carbon atoms in butyric acid can be made from lactic acid only by a synthesis; and the inference is obvious that the same synthesis occurs when the butyric acid is made from sugar, only in this case the lactic acid, if that is a necessary precursor, has to be made from the sugar first.

The nature of this synthesis was explained by Nencki, who suggested that the lactic acid broke down, as an α-hydroxy acid will, and as lactic acid itself is particularly prone to do, into the lower aldehyde, acetaldehyde, and hydrogen and carbonic acid. Two molecules of the aldehyde condensing to form aldol then give rise to a chain of four carbon atoms which by reduction and oxidation yields butyric acid:—

$$CH_3.C\begin{matrix}O\\H\end{matrix} + CH_3.C\begin{matrix}O\\H\end{matrix} \rightarrow CH_3.C\begin{matrix}OH\\H\end{matrix}-CH_2.C\begin{matrix}O\\H\end{matrix} \rightarrow CH_3.CH_2.CH_2.COOH$$

Now this reduction and oxidation are the same reactions that as we saw appear to underlie the formation of lactic acid and the saccharins from sugar, *the reduction of the β-alcoholic hydroxyl accompanying the oxidation of the terminal aldehyde group.*

The biochemical significance of the synthesis of butyric acid from lactic acid and from sugar by bacteria becomes much greater, however, when it is remembered that in this fermentation normal caproic is simultaneously formed, and as Raper showed also, though in still smaller amount, normal octoic or caprylic acid. The synthetic reactions by which butyric acid with four carbon atoms is produced appear therefore to be good also for the production of

acids with straight chains of six and eight carbon atoms, while the acids with five or seven carbon atoms in chain are not formed.

At this point, too, it should be remembered that the fatty acids with more than six carbon atoms which occur in nature produced by living organisms have almost without an exception an even number of carbon atoms and these atoms joined in a straight or normal chain. In butyric fermentation it seems that the reactions that lead to the synthesis of butyric acid may lead to the synthesis of acids with longer chains but still unbranched and containing an even number of carbon atoms, in other words, that these acids may be produced by condensation of two, three or four acetic aldehyde molecules. In higher organisms, plants or animals this same condensation carried further would result as Nencki suggested in the formation of the series of acids with straight chains of even numbers of carbon atoms leading up to palmitic and stearic acid. Normal octoic acid has been synthesised in the laboratory from acetic aldehyde by Raper. And in animals, in the fat of milk for instance, all the acids of the stearic series that contain an even number of carbon atoms are found, though some of them only in traces. Magnus Levy described the formation of butyric and acetic acids during the aseptic autolysis of the liver and gave evidence for his belief that their formation took place as in butyric fermentation from sugar. Hydrogen and carbonic acid were observed to be formed in these experiments as in butyric fermentation.[1]

Other experiments have been described in which the cells of the same organ under similar conditions formed acids of high molecular weight soluble in petroleum ether, apparently higher fatty acids. These results make it probable that the liver is the organ in which the synthesis of higher fatty acids is effected in animals (Leathes).

The doctrine that fats are products of the degeneration of proteins, which was long maintained, was supported by arguments which have mostly been shown to be misunderstandings of facts and into which it is not necessary to go. But to maintain that none of the material used in the synthesis of higher fatty acids in plants or animals is ever derived from a source in proteins would certainly be going too far. If the analysis given above of the probable nature of the synthetic processes involved when fat is made from carbohydrate is not altogether beside the mark, then it may be pointed out that lactic acid may certainly equally well be formed from alanine as from glucose, and that acetic aldehyde may not unreasonably be supposed to arise in the breaking up of other groups that can be traced back to an origin in proteins, of the aceto-acetic acid, for instance, derived through β-oxybutyric acid from leucine, tyrosine and phenylalanine. The experiments of Weinland with the larvæ of the blue bottle fly seem to supply a substratum of fact for such ideas. Even the juice expressed from the crushed larvæ, treated with peptone, was found to give rise to the formation of higher fatty acids.

[1] It seems probable that these results may have been due after all to the action of bacteria, a possibility which Magnus Levy himself acknowledged was not excluded. *Cf.* Wolbach and Saiki.

B. THE PHYSIOLOGICAL OXIDATION OF FATS.

The fats and oils are made use of both in plants and animals for the storage of reserve energy. In plants they are found in a large number of seeds, fruits and nuts stored in large quantities ready for the use of the embryo in germination. Hazel-nuts, walnuts and Brazil nuts, and the fruits of the olive and palm, contain from 50 to 60 per cent. of oil, sometimes even more than that. The seeds of flax, sunflowers, the cotton plant, firs, pines and cedar-trees contain from 20 to 40 per cent. Half or more than half of the food value of human milk, and of the milk of cows, is contained in the form of fat, and in some species of mammals a much larger proportion. Cow's milk and human milk contain about 4 per cent. of fat; the milk of sheep contains nearly twice as much; that of the reindeer and elephant four or five times as much, and porpoise milk as much, it is said, as 46 per cent. In the animal body fat is stored principally in the cells of the connective tissues, and in well-nourished animals amounts commonly to 10 or 12 per cent. of the body weight, more exceptionally to 20 per cent.; in one case in the dog, as much as 40 per cent. was found by Pflüger. It has been shown that this fat can be used as a source of energy as efficiently as either proteins or carbohydrates, and in starvation it is the stored fat that enables life to be maintained for many days. Under these conditions as much as 90 per cent. of the total energy developed in the body may be reckoned to come from the oxidation of fat. The heart, the organ that works hardest, contains in itself, including all the proteins of which it is itself built up, material the complete combustion of which would serve to run this machine for less than twenty-four hours, of which not more than about 5 per cent. is in the form of carbohydrate and as much as 30 per cent. in the form of fat.

The way in which the reserve energy stored in adipose tissue is put into circulation and conveyed to the organs in which the energy is liberated by the combustion of the fat is very little understood. It seems probable that the lipolytic enzyme found in connective tissues may in the first instance come into action: the transportation of fat across the barrier of a cell membrane requires the hydrolysis of the fat in the case of the intestinal epithelium, although the fatty acid and glycerine combine again immediately after. They are found combined in the lymph; and as is well known from the experiments of Munk, even when free fatty acids are given to a patient with a fistula of the thoracic duct they are found to have taken up glycerine and been converted into glycerides, since it is the glycerides that are found in the chyle of such a patient. But the transportation of fat from the connective tissues to the working organs is very difficult to study. The blood ordinarily contains about 0·2 to 0·3 per cent. of fat. But even in the case of a starving man, requiring, let us suppose, 2,000 calories per diem, and obtaining nine-tenths of this amount from his reserve fat, the blood would have to transport in twenty-four hours only some 200 grms. of fat. If the

total volume of the blood be taken at 5 litres and the circulation time at one minute, then the flow of blood through the body in twenty-four hours would be $5 \times 60 \times 24 = 7{,}200$ litres, and the average amount of fat transported by a litre of blood from the reserves to the working organs would be $\frac{1}{36}$ of a gramme or about 3 mgrms. by 100 c.c. It is clear from this that in those cases in which a demonstrable increase in the amount of fat present in the blood has been found, some other factor in addition to accelerated transportation of fat must be at work, and that the study of such cases can tell us little about the normal transport of fat.

The only point on which we can speak with certainty is that the mobilised fat, like the fat absorbed from the intestine, is, in the first instance taken up by the liver. For it can be shown that if animals are given a meal of fats with high iodine values the iodine value of the fat in the liver is raised as soon as enough fat has been absorbed; no such change can at this time be detected in the other organs of the body (Leathes and Meyer-Wedell). And on the other hand, it is a fact that in animals, that have not been treated with any special fat, the fat of the liver under ordinary conditions, like that of the muscles, heart, spleen and other organs, differs from that of the stored reserve fat in that it is composed of fatty acids with a much higher iodine value, commonly about 120 as compared with 40-60 in the adipose tissue (Hartley); while in all those conditions in which an active mobilisation of reserve fat can be shown to have taken place, the fat of the liver at once indicates that the stream of fat has been directed to this organ, by the low iodine value of the fatty acids of which it is composed. The fat in the other organs is not altered in this way, or not to anything approaching the same degree. In phosphorus poisoning, in delayed chloroform poisoning, as an effect of alcohol, of the action of mineral acids, of diabetes, phlorrhizine glycosuria or simple starvation, as well as in many other conditions, an active mobilisation of the fat reserves may occur or commonly does occur, and the liver cells are found loaded with fat of a low iodine value (Hartley and Mavrogordato, Leathes, Mottram). In some cases it has been shown that this fat comes from the connective tissues where fat is stored, not only by the alteration of its iodine value, but by its containing fat that is not usually found in the adipose tissue of the species, but which has by a special treatment been deposited in the connective tissues of the subject of the experiment. Lebedeff showed that the fat of dogs that after emaciation by starvation were fed on linseed oil contained linseed oil, and if such dogs were treated with phosphorus, the fat found in the liver also contained linseed oil.

Mobilised fat is therefore taken to the liver, where it can be detected easily in large quantities but not in other organs. The question, then, is, what is the purpose for which this transportation to the liver is necessary. There are two ways in which the fat found in the organs in which it is likely that fat is oxidised differs from fat that is merely stored and held in reserve in adipose tissue. As we have seen, it is composed to a much larger extent of unsaturated

acids as indicated by the iodine value. But in addition to this a large proportion of the fatty acids are combined with phosphorus and nitrogen. In the heart and muscles, in the kidneys and spleen as well as nervous tissues, lecithine and other compounds of fatty acids into which nitrogen and phosphorus enter are found in large amounts. Attempts have been made to estimate the extent to which the fat in these different organs is composed of these complex bodies. The methods employed make the results somewhat uncertain, but Rubow, for instance, calculates that at least a half of it is in the form of "lecithine". An idea of the changes which are brought about in the mode of combination of the fatty acids at some time after they have been mobilised and taken to the liver may be formed from the amount of them which can be obtained from the fat extracted from the adipose tissue on the one hand and the other parts of the body on the other. The connective tissue fat yields about 95 per cent. of fatty acids on saponification; it is composed therefore of very nearly pure glycerides. The fat extracted from the heart yields only about 60 per cent. The liver takes, as will be understood, an exceptional position; if it has not been recently loaded with mobilised fat, the fat in it is found to yield an amount of fatty acids similar to that yielded by the heart muscle and other organs, whereas if there is an excessive amount of fat in it the percentage of fatty acids yielded on saponification approximates to that which characterises a mixture of simple glycerides. It will be remembered that lecithine yields on saponification about 70 per cent. of fatty acids, and other phospholipines much less still, down to 40 per cent. A normally functioning liver keeps pace with its work and contains fat with a similar iodine value, and about the same percentage of fatty acids as that which characterises the fat of any active organ of the body in which fat is not merely stored in reserve in the inert unorganised form of the simple glycerides. But the liver, unlike other organs in the body or at any rate in quite another degree, may be found clogged with imported fat of a low iodine value and containing also a percentage of fatty acids indicating that it is mainly composed of as yet unaltered glycerides. It would seem therefore that with normal rates of fat mobilisation the liver fat is of a similar character with that found in the other organs of the body, but that when there is an excessive mobilisation of fat this may be revealed by the accumulation in the liver, alone of all the organs, of fat with a low iodine value mostly composed of simple glycerides and containing but little lecithine or other phospholipines. And it is a natural inference to suppose that not only the alteration in the fatty acids by which their iodine value is raised is effected in the liver, but also that combinations of these acids in which phosphorus and nitrogen occur are also built up in this organ; that it is the work of the liver not only to desaturate fatty acids, but also to combine them with phosphorus and nitrogen for the use of the body generally. These two changes, however, are apparently to some extent independent of one another. For it is possible to partially separate by means of alcohol, ether and acetone the simple and the complex

fats of the liver; and in that case it may be shown that a fraction containing but little nitrogen and phosphorus, and yielding on saponification nearly 90 per cent. of fatty acids, composed therefore only to a very small extent of phospholipines, may nevertheless have an iodine value that shows that the fatty acids have undergone desaturation although they are not yet combined with nitrogen or phosphorus (Kennaway and Leathes). We have much other evidence that the desaturation of fatty acids is the work of the liver. But this does not involve any prejudice as to the part borne by this organ in the synthesis of compounds of fatty acids with phosphorus; though this latter change must be effected in the liver it is quite possible that it may be carried out in other organs as well; when once the desaturated acids are supplied by the liver, the liver and each organ makes its own phospholipines for itself. On this point the evidence is not explicit, as the one change does not necessarily involve the other.

As to the desaturation of fatty acids in the liver the evidence, in addition to what has been cited above, is derived from a study of the fatty acids that have been isolated from the pig's liver by Hartley. The pulped tissue was heated with alkali till all was dissolved: the fatty acids were thrown out by the addition of sulphuric acid, converted into the lead salts and those soluble in ether separated. The unsaturated acids obtained from these were oxidised with permanganate in alkaline solution at a low temperature, and a dioxystearic acid was isolated differing in solubility and melting point from the one obtained in precisely the same way from pig's lard. This dioxy-stearic acid further oxidised by heating with permanganate was decomposed and yielded no pelargonic acid but caproic acid instead, and no azelaic acid, but evidence was obtained for the formation and presence of decamethylene dicarboxylic acid which, however, could not be isolated. The detection of these acids shows that the oleic acid in the pig's liver was different from the oleic acid in the pig's connective tissue, in having its unsaturated link between the sixth and seventh carbon atoms instead of the ninth and tenth. Since, also, the tetra-oxystearic acid obtained in the same way from the pig's liver appeared to be derived from an acid with unsaturated links between the sixth and seventh and the ninth and tenth carbon atoms, it seems probable that the new oleic acid in the pig's liver is formed not by an alteration of the position of the unsaturated link in the ordinary oleic acid, but by the introduction of an unsaturated link between the sixth and seventh carbon atom of stearic acid; the ordinary oleic acid brought to the animal's liver undergoes the same change and is so converted into the linoleic acid from which the above-mentioned tetra-oxystearic acid was obtained. The acids found in the pig's liver may be accounted for, in other words, by supposing that desaturation of stearic acid and of the ordinary oleic acid occurs by the introduction of a double link between the sixth and seventh carbon atom in each case. The evidence that Hartley obtained for the occurrence in the liver of a second linoleic acid, the constitution of which was not studied, may

in that case point to further desaturation of the oleic acid formed in the liver from stearic acid, by the introduction of a second double link not between the ninth and tenth carbon atom but in some other position in the chain.

We may argue, therefore, from the available data that there are certain positions in the chains of carbon atoms that constitute fatty acids which the liver attacks and where those chains are thus weakened. The fatty acids weakened in this way are then handed on to the other organs, and the subsequent course of the destructive changes by which these acids are made to yield to those organs their charge of chemical energy is determined by the positions of the weak links in the chains. What we know of these positions would lead us to expect caproic acid to be formed at any rate, and beside this dicarboxylic acids, malonic and probably others with larger molecules. If the dicarboxylic acids having an uneven number of carbon atoms give up CO_2, they will become lower fatty acids with an even number of carbon atoms; in this way the ordinary oleic acid, after the change brought about in it in the liver, may be expected to yield caprylic, caproic and acetic acids, and the oxidation of higher fatty acids resolves itself into the oxidation of lower ones of comparatively small molecular size. Now the oxidation of such acids as these, which it is known is readily effected in animals, has been illuminated by the experiments of Knoop. It was well known that benzoic acid and phenylacetic acid are excreted as hippuric acid and phenylaceturic acid, combined, that is to say, with glycocoll but otherwise unchanged. Knoop showed that higher homologues of these acids are excreted either as hippuric or phenylaceturic acid, having the fatty acid side chain shortened by the removal of two carbon atoms at a time, as long as that is possible, and after that suffering no further change except the condensation with glycocoll. Phenylpropionic acid in this way appears as hippuric, phenylbutyric as phenylaceturic, while phenylvaleric undergoing two successive β-oxidations appears as hippuric acid. This oxidation of the β-carbon atom in lower fatty acids has been confirmed in animals by Dakin, and shown also to occur in vitro with all the fatty acids when they are acted on by hydrogen peroxide. The β-oxybutyric acid which is excreted in acetonuria, and in that condition is the precursor of acetoacetic acid and acetone, is derived in part from constituents of the proteins, leucine, and also tyrosine and phenylalanine; but part, and probably in most cases the larger part, is derived from butyric acid that has been formed by β-oxidation from caproic acid and higher members of the series. The experiments of Embden and Marx show that the conditions under which a formation of acetone in animal cells from butyric acid occurs, permit also of the formation of acetone from caproic and caprylic acids, but not from the intervening acids of the series with an uneven number of carbon atoms. And the amount of acetone which they found to be formed from caproic and caprylic acids, compared with the amount formed from the same quantity of butyric acid, agrees remarkably with the amount required by the hypothesis that these

acids give rise to acetone formation by first being converted by β-oxidation into butyric acid.

The conversion of butyric acid into acetone in acetonuria, for which there is much other evidence, and the same change observed under the conditions of Embden's experiments, does not mean, however, that this is the normal course of oxidation of this substance in the animal body. Butyric and β-oxybutyric acids do not appear as acetone in the urine of normal subjects, though they increase the amount of acetone in acetonuria. They are probably converted into acetoacetic acid in all cases, but in the normal organism this substance does not break down into acetone and carbonic acid as it does in acetonuria. And Embden and Michaud found that a fresh pulp of liver cells to which acetoacetic acid was added caused large quantities, up to 60 per cent. of it, to disappear without converting it into acetone. This change did not vary in amount with the oxygen supply, so is probably not an oxidation. But on the other hand they were not able to show an increase in acetic acid. Acetonuria, then, throws light on the oxidation of fats in animals inasmuch as it appears to be a disorder of the last stages in the breakdown of fatty acids. And it is remarkable how the conditions in which this disorder is commonly found are conditions in which another disturbance in the metabolism of fat is common, the failure of the liver in dealing with and forwarding to the rest of the body the fat which has been called up from the reserves. Acetonuria and fatty liver may both be associated with starvation, diabetes, phlorrhizine glycosuria in starvation in dogs, with the after effects of anæsthetics, in cyclical vomiting, in marasmus in infants and many other conditions. To sum up then, by piecing together what is probable with what is known of the chemistry of the processes by which the energy of fat molecules is rendered available in the animal body: the fat is transported to the liver, unsaturated unions are there introduced into the fatty acids, and possibly there, too, the complex compounds of fatty acids with phosphorus and nitrogen built up. The unsaturated products, which are next found in the cells of other organs throughout the body, break down, probably where the unsaturated links have been introduced, and the lower acids so formed, by successive oxidation at the β-carbon atom, break down further to molecules of the size of acetic acid, which are lastly completely burnt to carbonic acid and water.

In plants the utilisation of fats by the embryo during germination has been but little studied. Enzymes that hydrolyse the fats or oils are liberated when the growth begins, certainly in some species, probably in all. The changes in the oil of sunflower and of ricinus seeds during germination were studied by von Fürth and were found to consist in an increase in the saponification value, which means a fall in the mean molecular weight of the acids, or in other words the formation of lower fatty acids from higher ones, and in a lowering of the iodine and acetyl values, which indicates that it is the unsaturated linkages and the hydroxylated carbon atoms that have been the weak places where the cleavage has occurred. But

beyond these observations there is nothing known as to the metabolism of fats in plants.

That under certain circumstances fats may undergo changes in animals and possibly in plants too, the result of which is that carbohydrates are formed, has sometimes been argued. The evidence for such changes in animals is indirect, based on the fact that oxygen may be retained in the body and not be accounted for in the products of oxidation that leave it, that the respiratory quotient in other words is lower than is compatible with the complete oxidation of any of the three classes of food. Such a condition, during hibernation, for instance, would be explained if it were shown that fats containing about 10 per cent. of oxygen were being converted into carbohydrate with 50 per cent. or more. Pflüger's argument that in glycosuria following removal of the pancreas in dogs very large quantities of fat are converted into sugar, rests upon the assumption that none of the sugar can be formed from proteins, a view that does not accord with common observations and opinions. But it cannot be maintained that the formation of sugar from fatty acids is impossible; because sugar is synthesised by animals, and as it is not known how this is carried out, it is impossible to say that it is not by a condensation of some simple group that may arise in perhaps the last stages of the disintegration and oxidation of fats no less than of proteins. There must be stages in which both these classes of material are brought down to a similar condition, final common paths in metabolism; for the amino acids deprived of their amino groups are already lower fatty acids, and their subsequent fate can hardly be very different from that of lower fatty acids derived from fat. If, for instance, the synthesis of sugar in animals proved to be brought about by condensation of formic aldehyde, who could say that this was the penultimate product of the disintegration of proteins only and not of fats as well?

C. THE ROLE OF FATS IN VITAL PHENOMENA.

Leaving the more purely chemical aspects of the physiology of fats, we may turn finally for a moment to consider the part they bear in the presentation of the phenomena of life.

It is, of course, most conspicuously as a reserve fund of fuel for the growing and working cells that they are of importance. The storage of 100 calories in the form of fat may be effected in the space of about 12 c.c. in tissue weighing about 11 grms. The storage of 100 calories as glycogen is never effected in less than ten times that bulk of liver tissue weighing 130 grms., rarely probably in less than twenty times. The influence of gravity in the chemical economy of animals is often to be observed, as in the arrangements by which birds dispose of waste nitrogen without having to carry water to dissolve it, and in the same way the main part of the fuel reserve in all animals is carried in a form that is at once light and compact and necessitates the carrying of no more water than is necessary for other purposes.

But the substances out of which an organism is built up may serve in other ways than as mere fuel. Carbohydrates themselves may come to form an integral and essential part of the fabric of the living machine, without which the organism could not be held together; in plants especially this is the case, but also in large classes of animals in which an exoskeleton is relied upon for maintaining the organic cohesion of the whole. Fats and substances chemically related to them, in virtue of their insolubility in water as well as their general chemical inertness, are similarly capable of being put to many uses in the organisation of plants and animals. The use to which wax is put by the bee may serve as an illustration: in the cells of the honeycomb a concentrated solution of hygroscopic sugars is kept safe from dilution by the moisture of its surroundings and so too from invasion by lower forms of living organisms. In a similar way the waxes and fats secreted in the leaves of plants protect the underlying cells from loss or access of moisture, and also from the solvent action of the common enzymes. Biologically, still more significant are the fats and waxes in the bodies of certain bacteria. The fatty substances which form a considerable part of the bodies of tubercle bacilli not only exhibit a very low iodine value, but offer a remarkable resistance to measures that are commonly efficacious in saponifying fats (Bulloch and Macleod). And there are reasons for thinking that the vitality and power of resistance of such organisms is intimately dependent upon the properties of the fats in which their bodies are enclosed or with which they are impregnated.

In the higher animals, however, the bulk of the fabric of the machine is of a protein nature; not only the working parts, but the framework of every cell as of the whole organism itself appears to be built upon a basis of protein material.

Perhaps, however, it is because we are still too apt to concen-

trate our studies of the phenomena of life upon those types which are nearest to ourselves, and in the organisation of which a more liberal use of proteins is made, that there has been a tendency to look upon "protoplasm," living matter, as pre-eminently, if not exclusively, of a protein nature. Protein has, with the growth of more exact chemical ideas on the composition of living things, come to be almost the modern equivalent for the earlier "protoplasm". And fats and carbohydrates are regarded as merely so much unorganised matter put to sundry menial uses by the living proteins. But there are facts which seem to point to some of the fats at any rate as being indispensably built into the most intimate organisation of living matter. As Hoppe Seyler pointed out, "lecithine" and cholesterol are found hardly less universally distributed where the phenomena of life are to be observed than the proteins themselves; and it is only in comparatively recent times that indirect sidelights have come into view which bear upon the significance of this fact.

In the first place we may remember the work of Hans Meyer and of Overton on the phenomena of narcosis. The large group of narcotics which Schmiedeberg spoke of as the alcohol group, including, with alcohol, ether, chloroform and many other substances whose common pharmacological properties could hardly be supposed to depend on any common features in their chemical constitution, act as Meyer's experiments seem to show because of their physical solubility in fatty substances. Meyer measured the narcotic action of a number of these substances by determining the minimum concentrations which at certain temperatures caused complete narcosis of tadpoles. He determined also the coefficients of partition for the same substances and the same temperatures as between olive oil and water. And he pointed out that the narcotic action of the substances increases with the amount of them taken up by oil from water, as the figures given from his paper in the following table show:—

	Temp.	Coefft. of Partition.	Min. Concentration for Complete Narcosis.
Alcohol	3°	0·026	0·33M
„	36°	0·047	0·14M
Chloral	3°	0·053	0·02M
Monoacetin	36°	0·066	0·014M
„	3°	0·093	0·011M
Acetone	3°	0·146	0·33M
„	36°	0·235	0·14M
Chloral	36°	0·236	0·004M
Benzamide	36°	0·437	0·005M
„	3°	0·672	0·002M
Salicylamide	36°	1·400	0·0017M
„	3°	2·223	0·0008M

The one irregularity that this series of figures shows, the case of acetone, as he says, may be accounted for by the fact that lecithine and such substances as lecithine behave with this solvent quite differently from oil; oil dissolves in acetone and lecithine does not. And we have seen that it is particularly fats insoluble in ace-

tone that are characteristic of living cells. According to these results, and the view based on them and others by Meyer, the narcosis, the suspension of vital activity, which results from exposure to these substances, is due to the physical changes they induce in the relation between fats and the other constituents of the living matter. The fats, or more correctly the lecithine and phospholipines, are essential to the cohesion and physical constitution of the protoplasm, so that any interference with the physical state of these substances arrests the vital functions. The cement which binds the organised matter together is loosened by the solution in it of foreign substances, and it is the loosening of the protoplasmic cement that makes it impossible for the normal processes of life to be carried on.

Attempts to form a concrete conception of the physical relationship in the structural organisation of cells between the fats on the one hand and the other constituents of living matter on the other have not been successful in obtaining general recognition. Some have spoken of "lipoid membranes" as if the living cell itself were enclosed in a fatty envelope and accessible only to such substances as can permeate this envelope through chemical affinities with the fatty material of which it is composed. Others, with at the present time more show of reason, are inclined to think of protoplasm as an emulsion of proteins and "lipoids". Loeb and v. Knaffl Lenz find that sea urchin eggs are liable to undergo cytolysis under the action of any process chemical or physical that causes the cell fats to become more fluid; in this cytolysis the cell membrane may remain intact; the disturbance is due to the alteration in the physical state of membranes, if that is a helpful conception at all, which are of a different texture from that conveyed by the idea of a cell wall, pellicles investing droplets that are so fine as to give a perfectly homogeneous appearance under the microscope.

There is no phenomenon which has been in recent years more studied than the phenomenon of hæmolysis. The red blood corpuscles are next to the bones, teeth and fat corpuscles the structures which contain a larger amount of solids than any in the body. The muscles, the liver and the kidney contain about 75 per cent. of water and 20 per cent. of proteins; the red blood corpuscles contain from 30 to 40 per cent. of a single protein, hæmoglobin, taken by itself. All this may be dissolved out of the corpuscle, and what is left, the stroma, retain the shape and appearance of the normal corpuscle before the hæmoglobin was removed. There are many facts which have been held to warrant the idea that in this corpuscle the hæmoglobin is enclosed in a sac or envelope of some fatty material. The formation of ice crystals results in hæmolysis; by it is assumed laceration of the enclosing membrane, just as corpuscles which are rubbed mechanically with powdered glass discharge their contents; and if the osmotic pressure of the fluid in which they are suspended is lowered they can be seen to swell and finally to burst, dilution of blood with water causing in

this way hæmolysis. It was pointed out in 1866 by Hermann that fat solvents, ether, chloroform and alcohol, as well as solutions of bile salts, all cause hæmolysis. But the idea of a single continuous membrane investing a corpuscle and forming a sort of cell wall within which is contained a strong solution of hæmoglobin, does not fit well with all the facts, and is probably not seriously held at the present day, though it is impossible to say that it has given place to any alternative and equally concrete conception.

And yet the role of fatty substances cannot be and has not been lost sight of. The fact that blood shaken with olive oil is laked does not mean merely that the oil dissolves off the surface membrane that confines the hæmoglobin within the corpuscle. The effect is not obtained if pure triolein is used. The oil must be partially hydrolysed, and then oleic acid or its alkaline salts are intensely active in causing hæmolysis. No more than 0·05 mgrm. of ammonium oleate is necessary to hæmolyse the corpuscles in 2 c.c. of a 5 per cent. suspension of ox-blood corpuscles (Noguchi). A fat or fatty substance that is itself without hæmolytic action becomes active if treated with a solution of lipase, and Neuberg has noted the frequency with which lipolytic enzymes are found side by side with hæmolytic substances, in the poison of bees, toads, snakes, and in certain bacteriolytic or cytolytic sera. The hæmolysis and anæmia that accompanies the presence in the intestine of the parasitic worm *Bothriocephalus latus* has been interpreted in connection with the facts that an ether extract of the dried worm is hæmolytic, that this property resides in the cholesteryl oleate or the oleic or other unsaturated acids that it contains, and that if dogs are given by the mouth cholesteryl oleate the cholesterol is not absorbed, but hæmolytic soaps are found in the chyle (Faust and Tallquist).

The poison of the cobra has active hæmolytic powers, but corpuscles of certain animals washed free of serum are not acted on by it unless something that can be obtained from the serum, which is soluble in ether or in alcohol, be added. And since "lecithine" makes the poison act on washed corpuscles, Kyes regards the hæmolytic agent as a combination of the venom with the lecithine present in the serum. Exactly how the lecithine is related to the resulting hæmolysis much discussion and experimentation has not made clear. It is interesting and remarkable that the hæmolytic action of saponine (Ransom) and also of agaricine and tetanolysine (Noguchi) is arrested by cholesterol; the saponine combines with cholesterol, and the combination is unable to act on the corpuscle and bring about its hæmolysis. It is apparently the alcoholic hydroxyl in cholesterol, not the unsaturated carbon atoms, that is here operative (Hausmann).

It is not, however, by any means entirely on account of the light that it has been hoped might be thrown on the physical constitution of protoplasm that the phenomenon of hæmolysis has been so assiduously worked at in recent years. When blood corpuscles, certain bacteria, animal cells or merely protein substances are injected into an animal, something is found to be formed in that

animal which reacts with similar corpuscles, bacteria, cells or proteins. An "antibody" corresponding to the particular "antigen" is found in its serum, which is specific for the particular antigen used.

It is in this way that immunity to a disease may in many cases be acquired after an attack of that disease. The antibody produced in the course of a disease, cholera or typhoid, is like the hæmolysin produced by injection of red blood corpuscles, composed of two factors: one specific for that kind of corpuscle or bacteria and not affected by moderately raised temperatures, and the other, the complement as it is called, the same probably in all animals and in all cases, and destroyed by heating to 56° C. for half an hour. This close similarity between the acquirement of hæmolytic powers and the acquirement of immunity to many diseases has concentrated on the study of hæmolysis much of the work that is aimed at the elucidation of the largest problems in pathology. It has been very widely held that all antigens must of necessity be of a protein nature. The obscurity of the reaction seemed indeed to acquire a certain familiarity, if not to be illuminated, with the belief that it was peculiar to proteins, the molecules of which are of such complexity that all mystery attaching to them ceases to be mysterious.

But in the case of the red blood corpuscles it appears that the specific antigen is not a protein, but a fat. Bordet showed that it was contained in the stromata: Bang and Forssman traced it down to a component of the ether extract of the stromata, which is insoluble in acetone, and even in boiling 90 per cent. alcohol, but soluble in benzene. This can hardly be a protein and must be related to the fats. It cannot, however, be a simple glyceride, nor cholesterol, nor even lecithine itself; though its insolubility in acetone points to its being a body of that nature. It is quite insoluble in water or salt solution, but unlike the great majority of antigens does not lose its antigenic properties if boiled for one or two hours with weak alkalies or acids.

In the course of their work Bang and Forssman made another important observation: the antigen is not itself the substance which combines with and neutralises the hæmolytic substance in the serum of the animal that has been treated with the antigen. This other substance is also present in the ether extract of the stromata and can be separated by the above solvents from the antigen; it is finally obtained in a form which is soluble in all fat solvents, but also gives a clear solution in aqueous solutions of sodium chloride. This separation liberates the phenomenon of hæmolysis once for all from the domination of the theory of the nature of immunity, which has for the last decade held in its grip the imagination of nearly all who have worked on these subjects. That it deprives it of all relation to the phenomenon of immunity is not implied nor likely.

The discovery of Bang and Forssman that the antigen which starts the production of hæmolysin is of the nature of a fat raises the fats to a level of biological significance to which hitherto only proteins have been held to attain. But the antigen which they found is, from the way in which it behaves with organic solvents,

probably one of the complex compounds containing nitrogen and phosphorus. Its constitution is unknown, and a respectable obscurity is assured for it probably for some time to come. Certain other facts have come to light which show that fats may be capable of setting up biological reactions such as previously were ascribed only to proteins; and in these cases it seems possible, from the scanty knowledge that we possess, that the highest biological dignity may have to be attributed to fats even that contain no nitrogen or phosphorus.

Deycke Pasha found that cultures of a streptothrix, which he obtained from an advanced case of leprosy, produced remarkable reactions in lepers into whom they were injected. The streptothrix is not the cause of leprosy, but like the leprosy bacillus and the bacillus of tuberculosis it stains with carbol-fuchsin and retains the stain after treatment with 25 per cent. sulphuric acid. This "acid fastness" is known to be due to fats or rather fatty acids with which the bodies of the bacteria are permeated. The reactions set up in the lepers were various in different cases, but sometimes beneficial, and especially so in those cases in which the organisms injected were particularly rich in the "acid-fast" material. In that case one of the results was that the bacillus lepræ from the leprous growths of the patients was found to have become less "acid fast". Accordingly attempts were made to obtain the fatty material from the streptothrix. Growths of this organism were washed and dried and extracted with ether till all the acid-fast substance was dissolved out, and the ether extract freed from impurities finally yielded a white waxy material to which the name nastin is given. This yields on saponification glycerine, and none of the higher alcohols such as are found in the extract of tubercle bacilli. It is spoken of by Deycke and others who have worked at it as being a simple fat, not a complex body like lecithine. When this substance is injected into a leper bacteriolysis results in the leprous growths, and a chronic moderate febrile reaction accompanies the treatment. This is ascribed to the absorption of the proteins of the bacilli, which, now that they are no longer protected by the acid-fast fat, are subjected to the solvent action of the proteolytic enzymes of the patient's leucocytes and tissue cells. Now the injection of nastin causes a reaction, too, in tuberculous patients; its use indeed in such cases is apt to be dangerous owing to the high toxicity of the proteins of the tubercle bacilli. But in strictly localised affections, tuberculous disease of a joint for instance, remarkable and most satisfactory results are said to have been attained. In animals some degree of immunity to tubercle has been conferred by injections of nastin. It has not been possible to isolate nastin from tubercle bacilli, but this is thought to be due only to the technical difficulties, and it is believed to be present in them nevertheless. For a leper that is not affected with tubercle if he receives injections of the substances contained in the tubercle bacillus, including those of a fatty nature, reacts as he does to nastin, though this is not the case if he receives only the proteins of the tubercle bacillus.

Finally the most delicate of all tests by which, what is called the "biological reaction" of an organism to injected substances, the formation, that is to say, of specific antibodies for these substances can be established, is what is known as the reaction of fixation of complement. As we have seen, the action of antibodies is known to depend on two factors: the antibody produced in an animal when it reacts to an antigen does not itself act directly upon the antigen, but it acts by enabling a third substance to act, which was present in the subject before, but by itself and alone was inefficient. Now the complement used is the same in all cases and in all animals. When, therefore, an antigen and its antibody meet they engage at the same time a certain amount of complement, and that this is the case can then be shown, because that complement is then no longer available for another combination of a different antigen and its antibody; the complement is then said to be fixed, and the fixation of complement when an antigen is added to a fluid, if the result is properly checked by adequate controls, proves that that fluid contains an antibody for that antigen. To make a long story short it has been shown that the serum of a leper that has been treated with nastin shows fixation of the complement when mixed with an emulsion of nastin, contains in fact an antibody for nastin. In other words nastin, a fat, it is believed a simple glyceride, is thus shown to cause in the leper a reaction resulting in the formation of a specific antibody, a reaction which till recently was supposed to be peculiar to the proteins. Those who have worked with nastin give reasons for their belief that in the reaction to tuberculin a fatty substance produces the formation of an antibody, and find, too, that in lepers an antibody is produced for Chaulmoogra oil when they have undergone treatment with this vegetable product.

It may in this connection also be pointed out that the ether extract of the red blood corpuscles of one species is known to be toxic for animals of another species, though not as a rule for the same species. The poisonous substance dissolves in dry ether, but not in alcohol or chloroform, and can be removed from an emulsion in salt solution by shaking it with olive oil. And as is the case with other specific reactive substances of a fatty nature, it is not destroyed if, in the form of an emulsion in normal saline solution, it is heated to 100° C. (Lefmann).

Neither is it only as specific poisons that fats may acquire a new significance: it has also been shown that the point of attack of the poison of tetanus is a substance which has been described on an earlier page—one of the components of the myelin sheath of nerves, cerebrone. That the poison of tetanus acts upon the central nervous system is known, and that it reaches the nerve centres by travelling from the infected wound along the nerve trunks was shown by Meyer and Ransom. In the nerve centres it combines with some component of the nerve tissue, since it can be rendered innocuous if mixed with an emulsion of brain pulp (Wassermann and T. Takaki). And now finally it has been shown that when it is mixed with Thierfelder's cerebrone, 50 mgrms. of this substance neutralise

between two and three hundred lethal doses of the toxin. And the cerebronic acid, that can by hydrolysis be split off from cerebrone, a substance free from nitrogen altogether, is found to be three times as active as cerebrone itself (K. Takaki). This observation might well be of service as an indication that in the study of the functions of the nervous system it may be profitable to devote attention not only to the protein axis cylinders but in some degree also to the uses and significance of those fatty sheaths which are as yet so little understood.

From all these considerations it appears likely that the chemistry and physiology of fats will require to be more studied in the future than it has been in the past.

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